THE INFLUENCE OF VARIOUS ORGANS ON THE CATALYTIC ACTIVITY OF THE BLOOD.[1]

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The object of this work has been to study the influence which various organs exert on the catalytic activity of the blood. This has seemed essential since the knowledge thereby gained will assist materially in interpreting the results obtained by the clinical application of the test.

Literature.—The only researches we have been able to find concerning the effect of removal of various organs on catalase are the isolated ones of Battelli and Stern.[2] After bilateral nephrectomy, they found that there was no change in the catalase of the tissues at death. They also poisoned guinea-pigs and rats with phosphorous with the purpose of producing fatty degeneration of the liver and retarding the function of that organ. They found that there was no change in the catalase of the tissues of animals that had died acutely, but in those animals where the liver had undergone a fatty change, the liver showed a marked reduction in its catalytic activity, while the other tissues, blood, kidney, spleen, muscle, brain, and lung all showed an increased activity. They found the same augmentation, though to a much less degree, in the other tissues of the frog after extirpation of the liver. On the basis of this work they concluded that catalase is not formed in a single organ, but that it is produced by various tissues which have the power of supporting each other in preserving the high catalytic activity of the body; that it does not play an essential rôle in the special function of one tissue, but that it fulfills a general function in which all the organs are interested.

While we have been unable to confirm the isolated findings of Battelli and Stern concerning the catalase of the blood and other

[1] Received for publication November 29, 1909.

[2] *Arch. di Fisiol.*, 1905, ii, 471.

tissues after nephrectomy, etc., their theory, with certain minor additions, is apparently supported by the results which follow.

The Relation of the Kidney to the Catalytic Activity of the Blood.—It has been shown previously that as a result of unilateral nephrectomy in rabbits there is a fall in the catalase of the blood; this fall, however, is only temporary, while after removal of both kidneys the catalase of the blood decreases until death and then the power of the tissues to split hydrogen peroxide is likewise found to be decreased. The same decrease was found in human tissues obtained from individuals dead of uremia, and in the blood and tissues of animals suffering with chronic nephritis, etc. It seemed, therefore, that the decline of the catalytic activity of the blood might be an index of the functional sufficiency of the kidney. To investigate this problem further we have in several instances repeatedly removed portions of both kidneys of a rabbit to determine if possible whether there is any change in the catalase of the blood if only the minimal amount of functioning kidney substance necessary to prevent the succession of uremia is left.

Rabbit No. 9.—In four successive operations the whole of the right kidney and three-fourths of the left were removed. The animal died about four months after the first operation, or a month after the last. At autopsy a piece of renal tissue about 1 cm. in its largest diameter was found. This showed at either end a large healing scar, leaving a piece of kidney which represented approximately one-eighth of the original amount of renal tissue.

The urinary findings during life were unimportant except that following each operation there was some albumin in the urine. The cause of death was unknown. The animal had gotten lean and weak during the last two weeks, but the urine remained free of albumin and casts. Fig. 1 represents the catalytic activity of the blood as determined in the first fifteen seconds[3] (solid dots), and the urine in cubic centimeters (hollow dots).

The curve (Fig. 1) shows that while there may be a temporary fall in the catalytic activity of the blood after total or partial nephrectomy, this fall may vary within wide limits (see first and fourth operations); that following the fall there is a compensatory rise which continues; that even a small amount, one-eighth of the original kidney substance, if actively functioning, is sufficient to keep the catalase of the blood at a high level. Therefore,

[3] The method previously described was employed (Winternitz, *Jour. of Exper. Med.*, 1909, xi, 200).

clinically with cystic kidneys, hydronephrosis, pyonephrosis and even with small contracted kidneys of chronic nephritis, where there is enough active renal tissue remaining to perform the function of that organ, there will be no fall in the catalytic activity of the blood.

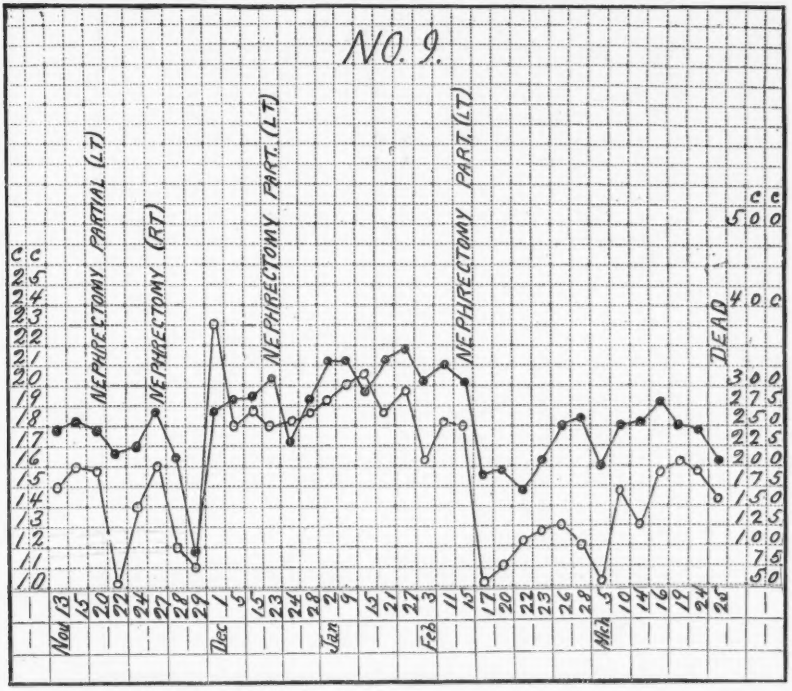

FIG. 1.

The Influence of the Thyroid on the Catalytic Activity of the Blood.—The experiments to be recorded here are divided into four groups: (1) Complete thyroidectomy. (2) Incomplete thyroidectomy. (3) Feeding of thyroid extract to animals whose thyroids have been completely removed. (4) Feeding of Thyroid to normal animals.

Rabbit No. 23.—The thyroid was completely removed and the animal, a young active buck, soon began to show the usual signs of thyroid insufficiency.

He became drowsy and fat, and his coat was no longer smooth. A month and a half after operation he was sacrificed and though careful search was made, no thyroid tissue could be found.

Fig. 2 represents the catalytic activity of the blood as determined in the first fifteen seconds.

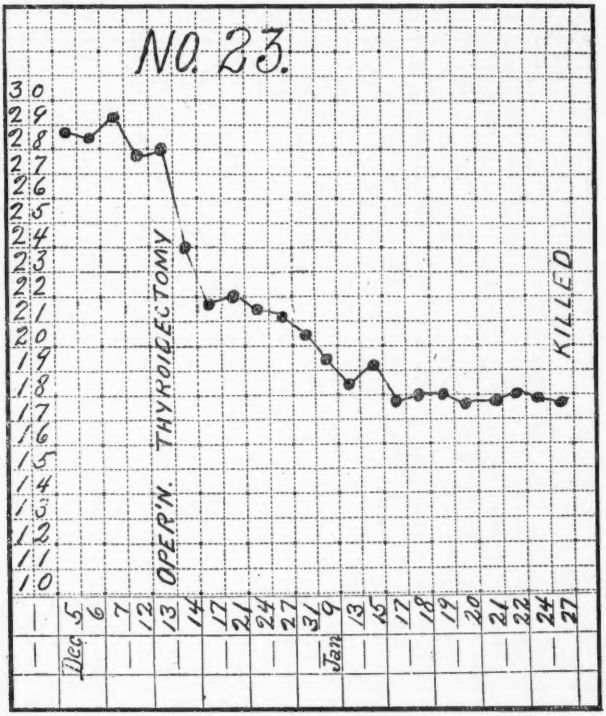

FIG. 2.

We have repeatedly confirmed the curve shown in Fig. 2. After complete thyroidectomy there is invariably a marked gradual decline in the catalytic activity of the blood. This varies slightly in extent, and at times several days may elapse after removal of the gland before the change occurs. The activity of the blood exhibits no tendency to increase even for months after operation.

Rabbit No. 21.—An attempt was made to remove the entire thyroid but when the animal was sacrificed over a month later a piece of tissue about 0.5

cm. in diameter, found in the region of the isthmus, proved to be thyroid. The alveoli were large; their epithelium was high, and in places was slightly folded toward the lumen. The amount of colloid seemed to be less than normal.

Fig. 3 shows that a small piece of thyroid tissue is sufficient to prevent the fall which follows total removal of the gland. While there was a slight fall lasting during the first five days after opera-

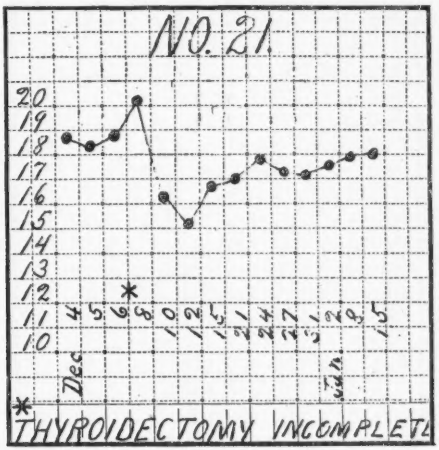

FIG. 3.

tion, this was rapidly compensated and the activity again remained stationary at practically the normal level.

The Influence of the Feeding of Thyroid on Thyroidectomized Rabbits.

Rabbit No. 43.—A large healthy animal showed, following removal of the thyroid, a marked fall in the catalase of the blood. It likewise manifested the usual changes of myxedema which, however, rapidly disappeared when thyroid was given by mouth. The animal became lean and active for a short period, but soon after the feeding was discontinued it relapsed into its previous lethargic condition.

Fig. 4 typifies the results obtained when thyroid extract is fed to thyroidectomized animals. After the drop in the catalytic activity of the blood following the removal of the gland, the curve will again rise if the animal be fed thyroid extract, but, as we have repeatedly shown, no matter how much of the gland be fed, the

curve will rise only to the level assumed before operation. Even if the drug be increased to such an extent that the animal becomes markedly emaciated and nervous and has a severe diarrhea, the catalytic activity of its blood will not rise higher than it has been

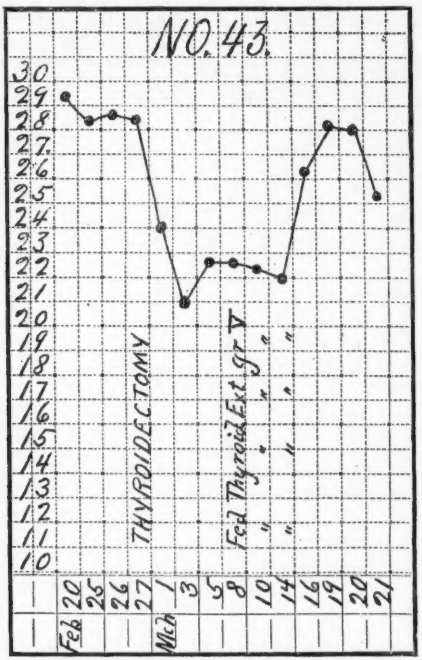

FIG. 4.

before removal of its thyroid. The same result is obtained if iodothyrin is used instead of thyroid extract.

What is the Effect of Feeding Thyroid Extract to Normal Rabbits?—Fig. 5 shows that feeding of thyroid extract to normal rabbits is entirely without effect on the catalytic activity of their blood. This observation may be expected, for even excessive feeding of animals with the thyroid removed fails to raise the catalytic activity of the blood above the normal level.

There is a marked fall in the catalytic activity of the blood fol-

lowing thyroidectomy. This decrease is permanent, provided the gland has been entirely removed, but if a portion is left behind the fall will be less and will not be permanent. If thyroid extract be fed to a myxedematous rabbit the catalytic activity of the blood will rise to the level it had before the thyroid was removed; it will remain at this level and will not rise higher, regardless of

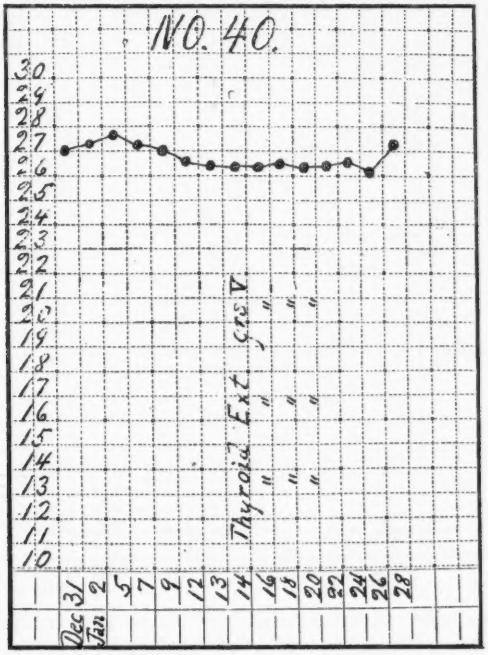

FIG. 5.

the amount of thyroid fed. Likewise, feeding thyroid extract to normal rabbits is without effect on the catalytic activity of their blood.

The Influence of the Spleen upon the Catalytic Activity of the Blood.—In a series of six rabbits from which the spleen had been removed, the results obtained were quite uniform.

Rabbit No. 50.—Splenectomy.

Fig. 6 shows that after removal of the spleen there is a gradual temporary fall in the catalytic activity of the blood which reaches a maximum usually within a week after operation; in another week the activity again rises to the level observed previous to operation. In some instances the fall may not be so great, while in others it may be more marked, and in some instances, after the

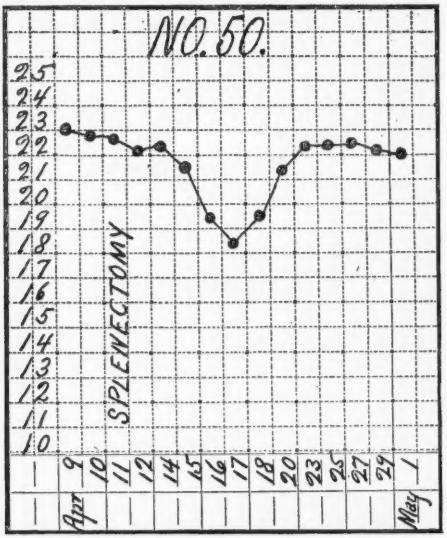

FIG. 6.

fall, there may be a rise in the activity to a level several points higher than it has been previous to removal of the spleen.

The tissues of the body apparently adjust themselves to the loss of the spleen and after a short interval succeed in preserving the normal catalytic activity of the blood. The change may resemble that following unilateral nephrectomy, and the tissues may overcompensate to a certain degree; the power of the blood to split hydrogen peroxide may be greater than before the removal of the gland.

The Influence of the Testicles and Ovaries Upon the Catalytic Activity of the Blood.—Since the results of removal of testicles

and ovaries have been almost identical, it will be sufficient to consider them together. The results have not been quite uniform. In all, twelve animals were used for the experiments on these organs. The experiments divide themselves into the following groups: (*a*) In three instances (castration twice, ovariectomy once) there was absolutely no change in the catalytic activity of the blood. The animals remained normal in appearance. (*b*) In all of the other cases following castration or ovariectomy there was a

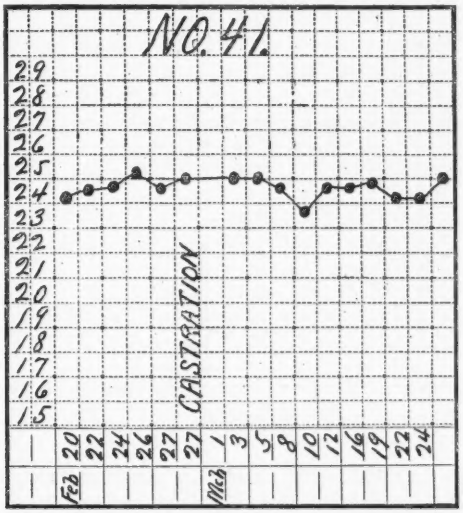

FIG. 7.

fall in the catalytic activity of the blood, which began within forty-eight hours after operation and continued during from seven to ten days, when there was again a compensation. In most instances this compensation was complete, while in one there was a slight overproduction, so that the catalytic activity was ultimately higher than it had been previous to operation; in two instances the compensation was not complete and left the power of the blood to split hydrogen peroxide slightly less than it had been normally.

Fig. 8 exhibits typically the result of the majority of experiments. It shows the initial fall in the catalytic activity of the blood fol-

lowing ovariectomy and shows the gradual rise in this action to about the primary level.

Hardly any discussion of this phenomenon is necessary, since here the explanation given for the changes in the catalytic activity of the blood following splenectomy seems entirely satisfactory.

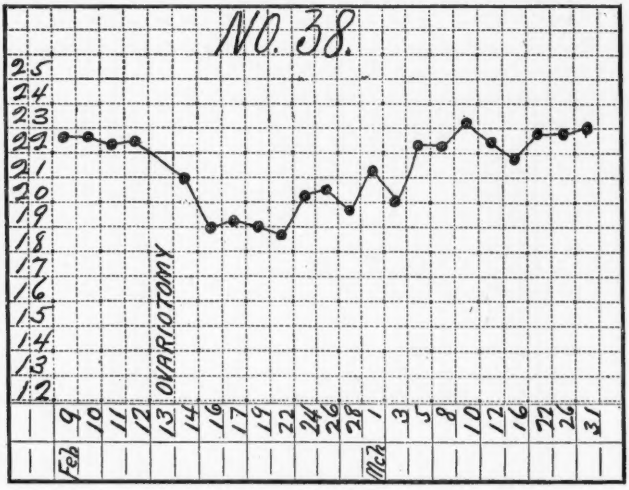

FIG. 8.

The Influence of the Liver on the Catalytic Activity of the Blood.—In this instance the only procedure which was found at all satisfactory was the removal at successive stages of different lobes of the liver.[4] In four instances, between 30 and 35 grams of liver were removed at the first operation. This had absolutely no effect upon the catalytic activity of the blood. In one of these animals 35 grams were removed at a second operation (see Fig. 9). Here again the blood retained its normal activity. We were unable to remove successfully more liver tissue and keep the animal alive for any length of time. However, in one animal where all but one small lobe was removed there was no change in the activity during ten hours before death.

[4] We are indebted to Dr. F. Ainley, of the obstetrical service, who kindly performed these operations for us.

Rabbit No. 54.—This animal withstood removal of two large lobes of the liver, but succumbed to the third operation. At autopsy 40 grams of liver were found.

The striking feature of the curve shown in Fig. 9 is its constancy. There is no material change in the catalytic activity of the blood on removal of large parts of the liver. Whether this absence of change is due to rapid compensation by other tissues of the body, or whether the liver has no influence on the catalytic action of the blood, we are unable to say. At any rate, we must remember that here only a part of the liver was removed, whereas in the experiments of Battelli and Stern the entire liver of frogs was removed and the entire liver of guinea-pigs was affected by phosphorous

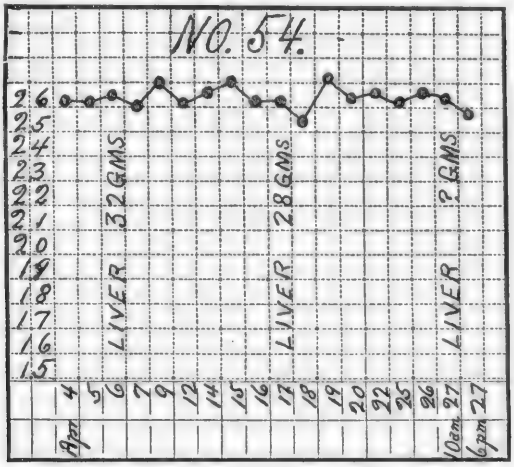

FIG. 9.

poisoning. It will be recalled that in these experiments Battelli and Stern found an increase in the catalytic activity of the blood and of other tissues.

The Influence of the Adrenal on the Catalytic Activity of the Blood.—The removal of one adrenal gland is without effect upon the catalytic activity of the blood. The removal of both glands is so unsatisfactory that we can only give the result of our experiments without drawing any definite conclusions. Of six animals,

where both adrenals were removed at the same operation, four died within a few hours. One lived for ten days and was then found disemboweled. On examination, the wound showed only the faintest signs of healing. (It seems worthy of note that the same result was obtained in another animal where one adrenal was removed at two successive operations.) The other lived for twenty-three days and was then sacrificed to see whether any adrenal tissue was present. None could be found. In both of these cases there was no change in the catalytic activity of the blood. In two other instances, however, although there was no fall in the catalytic activity after the removal of one adrenal, there was in both experiments a decided decline of catalytic activity after the removal of the second.

CONCLUSIONS.

1. *Kidney.*—After repeated removal of relatively large portions of renal tissue, when only one-eighth of the entire renal tissue remains, the catalase of the blood will show a normal activity if the part remaining functions actively. We expect therefore in human cases no change in this activity, even with advanced hydronephrosis, etc., as long as a small part of actively functioning renal tissue remains.

2. *Thyroid.*—There is a marked decrease in the catalytic activity of the blood following thyroidectomy. This decrease is permanent provided the gland has been entirely removed, but if even a small portion be left behind, the activity, although falling slightly at first, will soon reach the normal level again.

If thyroid extract be fed to a rabbit in which the catalytic activity of the blood has been reduced by complete thyroidectomy, this activity will rapidly rise to the level it had before operation, but will rise no higher. Likewise, if thyroid extract be fed to a normal rabbit it will be entirely without effect on the catalase of the blood.

3. *Spleen, Ovaries, Testicles.*—After removal of any of these organs, there is, as a rule, a temporary decrease in the catalytic activity of the blood, which usually reaches a maximum within a week and later ceases entirely, allowing the curve of activity of the blood to assume again its normal level within from ten to twenty days.

4. *Liver.*—Removal of relatively large portions of liver tissue is without effect upon the catalase of the blood.

5. *Adrenal.*—Removal of one adrenal has no effect upon the catalase of the blood. In two instances where the animals survived double adrenalectomy for any length of time the same result was obtained. In two other experiments where the second adrenal was removed some time after the first, there was a fall in the catalase of the blood.

We can support Battelli and Stern in their opinion that the catalase is not found in a single tissue but is produced by various tissues which have the power of supporting each other in preserving the high catalytic activity of the blood. (See spleen, testicles, etc., above.) They further conclude that the catalase does not play an essential rôle in the specific function of one tissue, but that it fulfills a general function in which all the organs are interested.

We may add that after suppression of the function of the thyroid there is a decline in the catalase of the blood, and after suppression of the function of the kidneys, there is a decline in the activity of the blood and tissues, which is not compensated. In the case of the kidney, we know that the organ itself is not responsible for the decrease, since the same result is obtained when the ureters are tied. With the thyroid the problem is more complex. It does not seem probable that the enzyme is formed to any extent in the gland, since the latter has only a slight capacity for splitting hydrogen peroxide; that the gland is intimately concerned in preserving the high activity of the blood is unquestionable and has interest especially in the light of the influence this gland has upon physical and mental activity.

CONCERNING THE DEPRESSOR SUBSTANCE OF DOG'S URINE AND ITS DISAPPEARANCE IN CERTAIN FORMS OF EXPERIMENTAL ACUTE NEPHRITIS.[1]

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PLATE IV.

Elsewhere,[2] in a discussion of the influence of kidney extracts upon the blood pressure, I have described the very striking depressor influence of dog's urine, when injected extravenously into other dogs. At that time this observation was of interest only in that it appeared to indicate that the similar depressor influence exerted by extracts of the dog's kidney was due to the content of urine which could not be removed.

My interest in this peculiar property of dog's urine was, however, again aroused by a chance observation made during the course of a recent study of diuresis in the pathological kidney. In this work, dog's urine, on account of its very decided depressor influence, from the effect of which the animal quickly recovers, was used as a means of rapidly lowering the blood pressure. For this purpose it was most satisfactory (Plate IV, Fig. 1) and never failed with a large number of normal urines. Early in the investigation, however, it was noted that the urine of an animal in the third day of a chromate nephritis failed to cause the usual depressor effect. This chance observation led to the routine investigation

[1] Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication November 19, 1909.

[2] Pearce, R. M., An Experimental Study of the Influence of Kidney Extracts and of the Serum of Animals with Renal Lesions upon the Blood Pressure, *Jour. of Exper. Med.*, 1909, xi, 430.

of the urine of animals with various forms of experimental nephritis. As a result, it was found that the depressor substance frequently disappears from the urine, or is greatly diminished, in those animals suffering from renal lesion characterized by extensive tubular injury and persists after the administration of substances causing only glomerular injury. This disappearance occurs from the third to the sixth day.

The following experiments illustrate this phenomenon as seen in nephritis due to potassium chromate and uranium nitrate:

Dog 17, weighing 9,800 grams, received 0.6 grams of potassium chromate on May 3; the urine of the following days contained albumin and an abundance of casts. Vomiting began on May 5. On May 6 the animal was used for the study of diuresis. Thirteen minutes after the administration of caffein, and at the height of diuresis 3 c.c. of the urine of the third day were injected intravenously. This caused a fall in pressure of only 7 mm., and when repeated after three minutes, one of 12 mm. Two minutes later the injection of the same amount of a normal urine led to a typical fall of 48 mm. of mercury.

It was this chance observation of the great diminution of the depressor substance in the urine of chromate nephritis that led to the study of the behavior of this substance in the urine of various forms of experimental nephritis. Other observations follow:

Dog 15, female, weighing 7,580 grams received 0.6 gram of potassium chromate on May 12. The urine was collected daily by catheterization and placed on ice. On May 13, the urine contained 0.9 grams of albumin per litre, by Esbach's method, and an abundance of casts. Vomiting began on May 14, and the elimination of nitrogen fell from a daily average of 3.98 grams to 1.84 grams; the urine contained 1.5 grams of albumin per litre. During the following days the dog refused food, vomiting continued and diarrhea developed. On May 17 the daily urines were injected intravenously.[3] The initial pressure was 84 mm. of mercury. The normal urine of May 12 caused a fall of 36 mm.; the urine of the first day of nephritis, a fall of 21 mm.; of the second day, 40 mm.; of the third day, 26 mm.; of the fourth day, 35 mm., and that of the fifth day, a barely percipitable drop of 6 mm. This injection was repeated after several minutes with the same result. An injection after three minutes of an equal amount of the normal urine of May 12, caused a drop of 39 mm.

Dog 20, weighing 6,640 grams, received on May 5, 0.015 gram of uranium nitrate, which caused the appearance of albumin and casts in the urine. On

[3] In these experiments the animals received, per kilo of body weight, one-third c.c. of 1 per cent. solution of morphine subcutaneously and 10 c.c. of a saturated aqueous solution of chloretone by stomach. The pressure was taken from the left femoral artery with mercury manometer. The injections were made into the right femoral vein, always in amounts of 3 c.c.

May 8, the animal received injections of its own urine as collected on May 5, 6, 7 and 8, and that of the third day (May 8) was found to be devoid of depressor substance. This urine was of light color with a specific gravity of 1,011, contained albumin (2.7 grams per litre by Esbach) and casts and reduced Fehling's solution. The initial blood pressure of this animal was 54 to 60 mm. of mercury which by the administration of adrenalin, caffein and salt solution, given intravenously, was raised after half an hour to 74 mm. of mercury. The injection of 3 c.c. of the urine of the third day caused no change in the pressure, while an equal amount of normal urine (May 5) produced a fall of 41 mm. of mercury.

In all the above experiments the urine was injected into the animal which had excreted it. In order to control the possibility of the condition of the animal's being a factor, the following experiments were made:

Dog 22, weighing 5,840 grams, in the second day of uranium nephritis, received 3 c.c. of the urine of Dog 20 (see above) which had lost its depressor power. The normal blood pressure of 80 mm. of mercury was not altered, whereas the dog's own urine, in the same dose, caused a fall of 40 mm. of mercury.

Dog 26, a normal animal, weighing 7,860 grams, received successively the urine of each day from another dog[4] suffering from a uranium nephritis. The normal urine of the day of injection caused a fall of 36 mm., that of the first and second days of nephritis, 36 and 30 mm., respectively, while that of the third day caused no depressor effect, but a very slight rise of 4 mm. This urine of the third day amounted to 340 c.c., had a specific gravity of 1,026, contained numerous casts, 4.8 grams of albumin per litre by Esbach's method, and reduced Fehling's solution. The urine of the fourth day, on which the animal died, caused a drop of 14 mm., while a final injection of the normal urine of May 9 produced a fall of 40 mm. of mercury.

Dog 40, a normal animal, weighing 5,720 grams, received the daily urine of Dog 35, with uranium nephritis. The urine of the sixth day failed to cause a depressor effect. The urine of this day amounted to 164 c.c., with a specific gravity of 1,006; albumin and casts were present, and the urine caused a reduction of Fehling's solution. The urine of each of the previous days caused a drop ranging between 40 to 44 mm. of mercury.

Dog 38, weighing 5,540 grams, with chromate nephritis, failed to show a complete loss of the depressor substance, though it was considerably diminished. Thus, first day, drop of 40; second day, 18; third day, 10; fourth day, 10; fifth day, 28; sixth day, 6; a final injection of the first day's urine caused a drop of 44 mm.

Dog 46 showed a decrease of less degree. This animal, 5,640 grams in weight, received 0.06 grams of potassium chromate subcutaneously. The normal urine previous to injection and that of each of the five succeeding days was

[4] Dog 23 received 0.015 gram of uranium nitrate on May 9. Urine of May 9, 10, 11, 12 and 13 was saved on ice.

obtained by catheterization and kept on ice. Injected into a normal dog, the control urine gave a fall of 68 mm. of mercury and the urines of first to fifth day, of 58, 46, 40, 30 and 28, respectively.

These observations demonstrate conclusively that from the third to the sixth day in chromate and uranium nephritis, the depressor substance disappears or is greatly diminished.

In some of the animals the disappearance of the depressor substance seemed to bear a relation to the onset of anuria. This is supported by the observation that when anuria preceded death the depressor substance was absent from the last urine voided. It would appear, therefore, that anuria may be preceded by an arrest of that function of the kidney which is responsible for the elimination of the depressor substance.

On the other hand, in those animals which do not succumb to the nephritis, large amounts of dilute urine may be voided and the depressor substance eventually reappears. This is illustrated in the following experiment:

Dog 48, female, 14,390 grams in weight, received subcutaneously 0.015 gram of uranium nitrate on October 15 and 0.0075 gram on October 16. The urine of each day was obtained by catheterization and placed on ice. The following table gives the examination of the urine passed voluntarily and the effect on blood pressure (Dog 52) of that obtained by catheterization (Plate IV, Fig. 2).

Date.	Urine.			Fall in blood pressure.	
	Amount.	Specific gravity.	Albumen (by Esbach).		
Oct. 15	340	1,038	0	40 mm. Hg.	
" 16	300	1,039	½ grm.	34 " "	
" 17	370	1,032	4 "	30 " "	
" 18	480	1,026	3½ "	0 " "	
" 19	800	1,020	1¼ "	0 " "	
" 20	750	1,017	1½ "	0 " "	(Control 48 mm.)
" 21	820	1,015	1½ "	0 " "	
" 22	450	1,018	1½ "	12 " "	
" 23	520	1,012	¾ "	4 " "	(Control 56 mm.)
" 24	880	1,010	½ "	16 " "	
" 25	—	—	—	4 " "	
" 26	720	1,013	½ "	30 " "	(Control 44 mm.)

The depressor substance disappeared on the third day and it did not reappear until the seventh day, increasing somewhat on the ninth to eleventh days. The reappearance corresponds roughly to

the decrease of albumin and may, therefore, be an indication of the gradual return of the kidney to normal.

The increased volume and low specific gravity of the urine in such experiments suggested the possibility of the influence of dilution. To control this, diuresis was produced in normal dogs by the use of caffein and saline solutions and the depressor effect of such urine determined. It was found that diuresis does diminish the depressor power but never causes its disappearance. Thus, in one animal, the bladder urine collected before diuresis caused a fall of 64 mm., while the urine at the height of diuresis caused a drop of only 30 mm. In a second dog the depressing power of the normal urine was 60 mm.; of the urine of the early stage of diuresis, 32 mm.; and that of the height of diuresis, 16 mm. The mean blood pressure for these respective observations was 117, 112, and 129 mm. of mercury.

The results in a third animal may be presented as follows:

Dog 52, male, weight 8,480 grams, morphine and chloretone anesthesia, cannula in bladder, pressure from right femoral artery, injection into left femoral vein, each injection 3 c.c.

Urine collected.	Blood pressure.	Fall.	
11.10–11.35	110	40	
11.39			20 c.c. 1 per cent. caffein solution in vein.
11.40–11.50	110	20	Urine slightly yellowish. Rapid flow.
11.50–11.55	118	24	Urine water white, flow very rapid.
11.55–12.05	120	20	Flow diminishing and yellow color returning.

It is thus shown that although diuresis may, by dilution, diminish the depressor substance in a given amount of urine, it does not cause its disappearance. Diuresis cannot, therefore, explain its absence in the urine of tubular nephritis.

In definite contrast to the urine of tubular nephritis is that of vascular nephritis due to arsenic or cantharidin. No diminution of the depressor substance could be demonstrated in the urines of six arsenic and six cantharidin animals under observation for periods of from three to six days. These observations would appear to demonstrate conclusively that the depressor substance is eliminated

by the tubular epithelium. The diuresis experiments point to the same conclusion.

Many efforts have been made to determine the nature of the depressor substance, and although it has not been possible thus far to isolate it in pure form, the observations which have been made are of some interest.

Boiling the urine for a few minutes does not destroy the depressor substance but any form of prolonged heating, as boiling for an hour or more, simple distillation, evaporating to dryness on a water bath, ashing, or extracting by alcohol with the aid of heat, causes its disappearance either completely or to such an extent that the usual injection of 3 c.c. produces a drop of only 6 to 8 mm. of mercury.

As the application of heat was thus shown to be undesirable, various methods at room temperature were tried. An attempt to separate the salts from other constituents of the urine by precipitation with lead salts gave negative results. Neither the filtrate nor the precipitate, after treating with hydrogen sulphide, contained the depressor substance, or at least if present, its effect was but slight. Dialysis caused but little change in the depressor substance; after four days of dialysis, the effect produced was as great as that caused by the normal control urine, but after eleven days it had lost three-quarters of its power. Thus the control urine caused a fall of 44 mm.; that of the fourth day of dialysis, a fall of 40 mm., and that of the eleventh day a fall of 12 mm. of mercury.

Many other procedures were attempted without success, until in the process of extraction by ether, alcohol and chloroform, it was found that it was possible to precipitate completely the depressor substance in an impure form by large quantities of alcohol.[5] Nine to ten volumes of alcohol well shaken with the urine causes a whitish precipitate to appear, which, on standing, settles out in very definite amount. This precipitate dissolves readily in distilled water or salt solution and in equal dose causes as great a fall in pressure as does the original urine. The filtrate, on the other hand, evaporated at room temperature, or at 37.5° C., and brought back

[5] It may also be precipitated by alcohol and ether mixture and to less extent by ether alone.

to original volume causes no effect or, in some instances, a very slight drop. By this procedure the depressor substance has been entirely separated from the urine, leaving an inactive filtrate. The precipitate thus formed does not, however, represent a single body or yet a group of bodies of like nature. Despite many attempts to remove impurities by repeated precipitation and washing, the precipitate contains both salts and protein, though in carefully prepared precipitates the latter reacts but faintly to the usual tests. Tests for sulphates and phosphates were always frankly positive and chlorides were present in faint traces. The biuret reaction and Heller's ring were faint, but phosphotungstic acid caused a precipitate, as did also picric acid in acid solution. The amount of nitrogen in various samples, as estimated by the Kjeldahl method varied from 0.006 to 0.009 per cent.

Concerning the nature and mode of action of the depressor substance, a survey of the literature offers no explanation. Bouchard,[6] who describes a variety of effects for human urine, and ascribes considerable importance to potassium, makes no comment on the effect on blood pressure, nor does Herringham,[7] who considers the toxicity of the urine in the healthy subject to be merely an " unexact expression of the excretion of potash." Beck[8] alone appears to have determined the effect of urine injections upon the blood pressure. The urine of healthy men injected into the rabbit causes, in his experience, little or no effect if small amounts are slowly injected. Rapid injection causes a fall in pressure with quick recovery while with large amounts the fall in pressure ends in death, due apparently to heart paralysis. Beck attributes the chief toxicity to potassium salts. No tracings are reproduced and the similarity of the effect I have observed to that of Beck cannot be judged. Injections of potassium salts, more especially the chloride, have not given in my hands the same effect as the urine and, moreover, the ash of dog's urine does not contain the depressor substance.

[6] Bouchard, Ch., Lectures on Auto-Intoxication in Disease, Philadelphia, 1906.

[7] Herringham, U. P., An Account of Some Experiments on the Toxicity of Normal Urine, *Jour. of Path. and Bact.*, 1900, vi, 158.

[8] Beck, A., Ueber die Giftwirkung des Harnes, *Arch. f. d. ges. Physiol.*, 1898, lxxi, 560.

As far as I have been able to cover the literature, the only depressor substances which cause a fall of the degree and character produced by dog's urine are certain of the protein bodies, as proteoses, peptones and like substances, as described by Chittenden[9] and his associates. In many instances these results are identical with those due to urine. Some, however, differ in that the return from maximum drop as, for example, that due to "auto-albumose" is much slower than after the injection of urine; the former in some instances not returning to normal for an hour, whereas the return with urine is a mattter of but a few minutes.

Another similar fall is that recently described by Biedl and Kraus[10] in connection with so-called anaphylactic shock. Working with dogs, they found associated with this condition a profound peripheral vaso-dilatation with correspondingly grave fall in blood pressure. They comment on the similarity of this effect to that caused by the injection of Witte's peptone.

These observations however throw no light on the mode of action of the depressor substance of dog's urine.

Certain facts, which I have here presented, are of importance in connection with the results of Bouchard's study of the toxicity of urine. Bouchard, in his early work, found that heating the urine diminished its toxic action and this he has recently again demonstrated in collaboration with Balthazard and Camus.[11] The study of the depressor substance here described has shown that heating diminishes its action and, if long continued, causes its entire disappearance.

Again, Bouchard states that he has observed the entire disappearance of the toxicity of the urine in individuals with uremia. He emphasizes this by stating that the disappearance of the toxic substances from the urine corresponds to the development of the

[9] Chittenden, R. H., Mendel, L. B., and McDermott, H. E., Papain-Proteolysis with Some Observations on the Physiological Action of the Products Found, *American Jour. of Physiol.*, 1898, i, 255; also, Chittenden, R. H., Mendel, L. B., and Henderson, Y., A Chemico-Physiological Study of Certain Derivatives of the Proteids, *American Jour. of Physiol.*, 1899, ii, 142.

[10] Biedl, A., and Kraus, R., Experimentelle Studien über Anaphylaxie, *Wiener klin. Woch.*, 1909, xxii, 363.

[11] Bouchard, C., Balthazard, and Camus, J., Influence du chauffage des urines sur la toxicité urinaire, *Compt. rend. d. l'Acad. d. sc.*, 1908, cxlvii, 662.

nervous manifestation of uremia and at such times, he states, the whole twenty-four hours urine has no more toxic effect than has distilled water. Purdy[12] also states that in some forms of acute and chronic nephritis, with changes which greatly cripple the functional capacity of the kidney, the urine may become almost non-toxic.

Whether or not we accept Bouchard's general principles, and despite the fact that he worked with unknown substances or combinations of substances, it is a matter of importance that the toxicity of the urine diminished with the onset of uremia; and this observation is of peculiar interest in view of my observaton that the depressor substance disappears from the urine at the height of functional incapacity and frequently this disappearance precedes the development of anuria or coincides with the gastro-intestinal disturbance characteristic of experimental uremia. Furthermore, I have found that whereas 5 c. c. of normal urine injected into the ear vein of a rabbit may cause immediate death, an equal dose of the urine of the same animal after the depressor substance disappears has no toxic effect.

The disappearance of the depressor substance from the urine presupposes that its retention in the body might operate to produce a low blood pressure. Of this there is some evidence. Not infrequently these dogs have a pressure of only 54 to 60 mm. of mercury, whereas normal dogs and those with experimental nephritis not characterized by disappearance of the depressor substance, usually have a pressure of 80 to 90 mm. of mercury. These observations cannot, however, be accepted as conclusive, for not only are they small in number, but occasionally this low pressure was not observed. On the other hand, arsenic and cantharidin dogs frequently have this low pressure without the disappearance of the depressor substance from the urine. It is possible that some other factor may be at work. To settle this point successive determinations from day to day on the same animal before the production of nephritis and during its various stages must be made by some indirect method.

[12] Purdy, C. W., Practical Urinalysis and Urinary Diagnosis, Philadelphia, 1902.

CONCLUSIONS.

1. Normal dog's urine contains a depressor substance which, when urine is injected intravenously in doses of 3 c.c., has the power to cause a fall in the dog's blood pressure, equal usually to 40 to 70 mm. of mercury.

2. The exact nature of this depressor substance has not been determined. It is not destroyed at 100° C., but does disappear on prolonged heating; it does not dialyze readily; it is completely precipitated from the urine by alcohol. The alcohol precipitate which contains a minute amount of nitrogen and various salts has a depressor power equal to that of normal urine.

3. The depressor substance disappears at the third to sixth day from the urine of dogs with tubular nephritis due to uranium nitrate or potassium chromate; or, if it does not disappear entirely, it is greatly diminished in amount.

4. This disappearance or diminution has not been observed in those forms of vascular nephritis due to arsenic or cantharidin.

5. The depressor substance would appear, therefore, to be associated with normal tubule function. This view is supported by the observation that it is greatly diminished at the height of diuresis in the normal animal. Its disappearance in experimental nephritis may be taken as an indication of extensive tubular injury and its reappearance as an indication of beginning repair.

6. The non-elimination of the depressor substance is accompanied in some animals by a low general blood pressure. This suggests an influence due to the retention of this substance, but this point has not been absolutely determined.

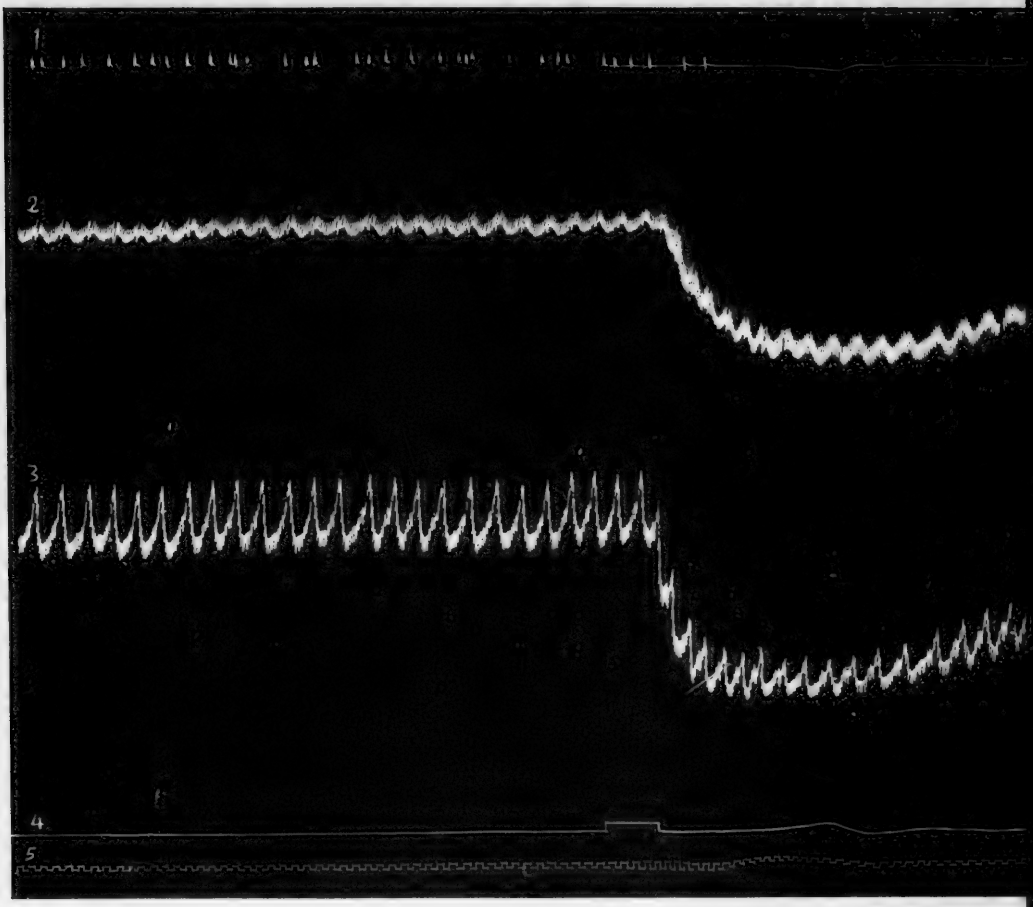
EXPLANATION OF PLATE IV.

FIG. 1. This tracing illustrates the depressor effect of 3 c.c. of normal dog's urine injected into the femoral vein of a normal dog (weight 7,980 grams), at the height of diuresis due to caffein and five per cent. salt solution (morphine and chloretone anesthesia).

1. Urine record (bladder cannula).
2. Oncometer record (left kidney).
3. Blood pressure (left femoral artery).
4. Base-line with injection record.
5. Time marker (seconds).

FIG. 2. (1) Effect on blood pressure of normal Dog 52 of 3 c.c. of urine collected by catheterization from Dog 48 previous to injection of uranium nitrate.

(2) Shows absence of depressor substance in urine (by catheterization) of Dog 48 on the fifth day of nephritis (see text).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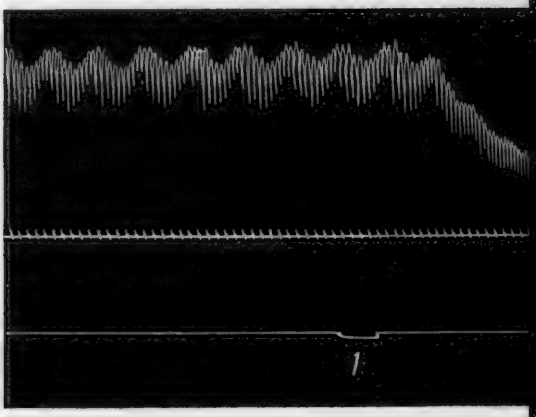
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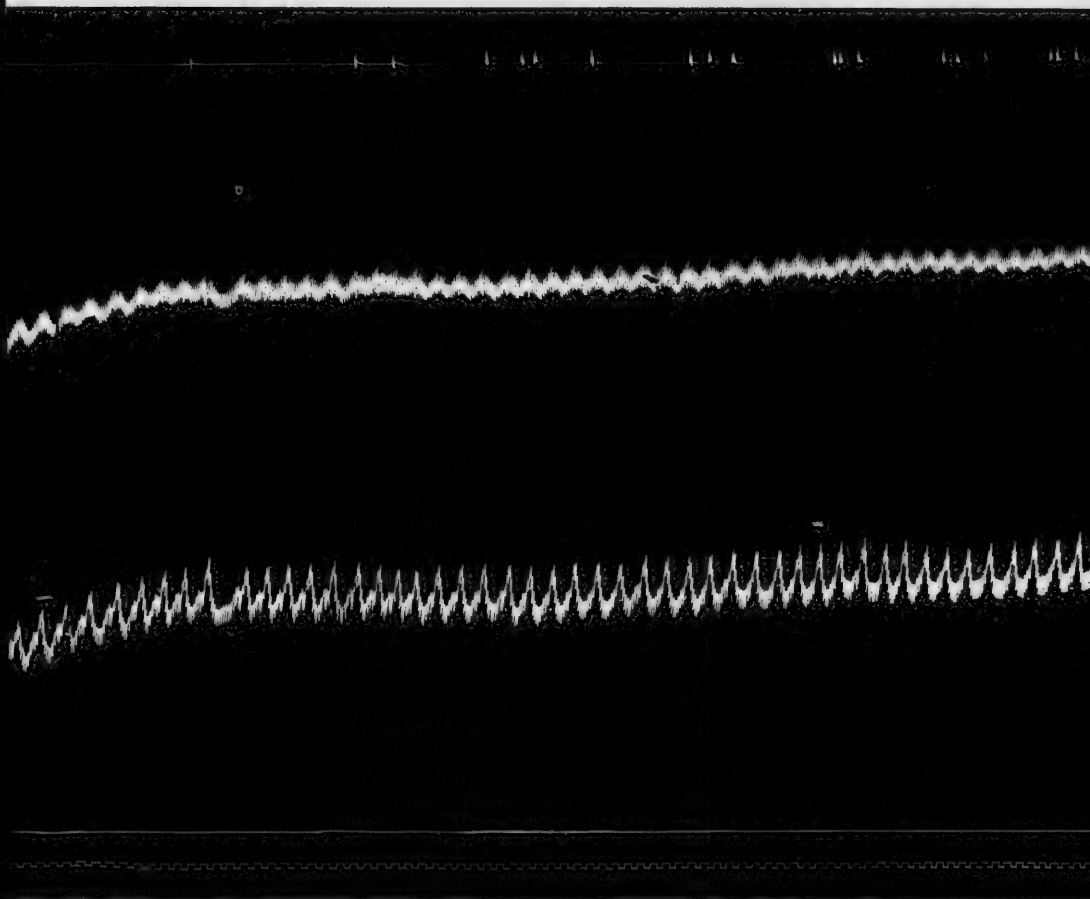

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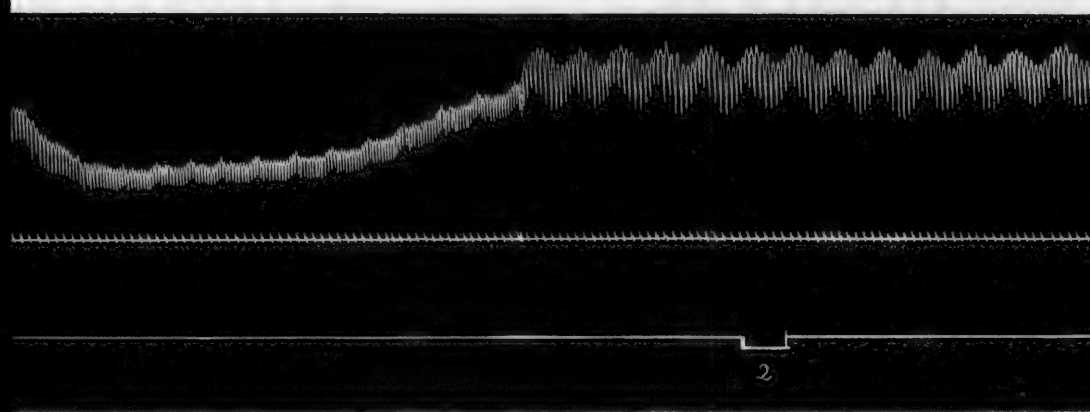

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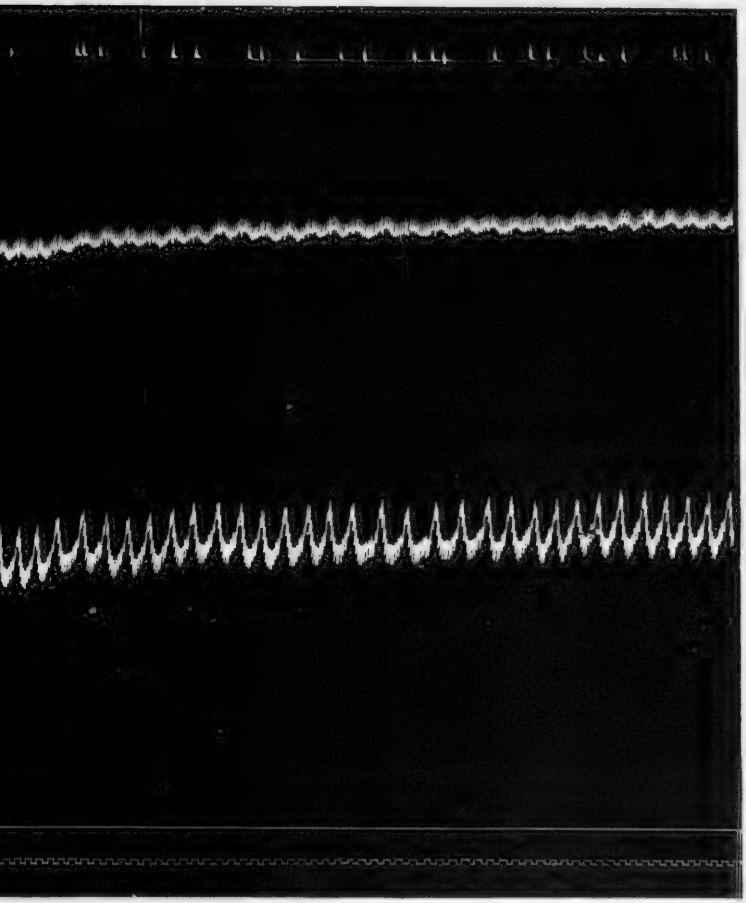

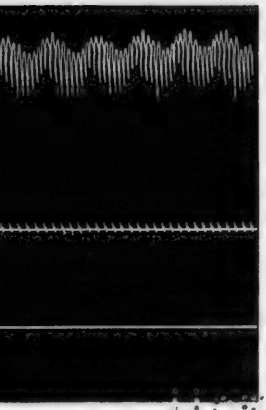

PERITONEAL PATCHING OF THE AORTA.[1]

By ALEXIS CARREL.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

PLATE V.

INTRODUCTION.

The peritoneal patching of an artery consists of substituting for a small part of its wall a patch extirpated from the abdominal wall and composed of peritoneum, sub-peritoneal connective tissue and transverse muscle. This operation was developed with the view of studying two points, surgical and biological, of the problem of regeneration of arteries in mammals. Can an opening through the walls of a large artery be safely occluded by a piece of peritoneum? How can an artery use heterogeneous anatomical elements in order to redintegrate itself, and by what method will the function recreate the organ?

TECHNIQUE.

I developed a technique for the patching of arteries in November, 1905, at the University of Chicago. Afterwards I performed with Dr. Guthrie some arterial grafts of small pieces of veins or arteries. This method was employed for the lateral implantation of small vessels on larger ones. We used it in some experiments of renal transplantations.[2] It has given also excellent results to Stich and Makkas[3] in the transplantation of the thyroid glands. The operation is composed of four stages: preparation of the graft, opening of the aorta and graft, and reëstablishment of the circulation.

1. *Preparation of the Graft.*—A rectangular peritoneal flap is resected, under ether anesthesia, from the antero-lateral wall of the abdomen. It is composed of the peritoneum together with the sub-peritoneal tissues alone, or with the transverse muscle. Then

[1] Received for publication December 29, 1909.

[2] Carrel, *Johns Hopkins Hospital Bulletin*, 1906, xvii, 236; Carrel and Guthrie, *Jour. of the American Med. Assn.*, 1906, xlvii, 1648.

[3] Stich und Makkas, *Beitr. z. klin. Chir.*, 1908, lx, 431.

the flap is washed in Locke's solution and preserved for a few minutes in vaseline.

2. Opening of the Aorta.—Hemostasis of the aortic segment, upon which the graft is going to be placed, is secured by two Crile forceps, and by ligature or forcipressure of the lumbar collaterals. The anterior wall of the aorta is opened, and a piece of it extirpated. The aortic wall is hard and friable, yet the incisions must be very neat and the edges must not be bruised. Very sharp scissors or a knife must be used. Half of the circumference of the aorta can be removed. The shape of the opening is triangular or oval. As soon as the vessel is opened the blood is washed out, and the edges are covered with vaseline.

3. Graft.—The patch is removed from the vaseline, and with the scissors it is given the same shape and dimensions as the opening of the aorta. It is fixed to the edges of the opening by four equidistant stitches. They are deep through and through stitches. In tying the threads care is taken to approximate accurately the vascular and peritoneal endothelia. If the muscle or the connective tissue overlaps the edges of the peritoneum they must be reduced to the proper length, for it would be dangerous to include them into the line of suture. Afterwards, the graft is completed by a continuous suture of the edges of the patch to the edges of the aortic opening.

4. Reëstablishment of the Circulation.—The lower Crile forceps is removed, while the suture is slightly compressed by a gauze pad. If, after a few minutes, there is still some hemorrhage, a few complementary stitches are added. The upper forceps is then removed and the circulation reëstablished. After that, the muscle is united to the connective tissue sheath of the aorta by a few stitches and the operation is ended.

EXPERIMENTS AND RESULTS.

Three experiments only were performed. In the first one the technique was developed. As I had no assistant, the operation was difficult. The animal died from the anesthetic.

I made the second experiment with Dr. Guthrie at the University of Chicago.

Under ether anesthesia, a small part of the abdominal aorta of a cat was extirpated and replaced by a peritoneal flap taken from the anterior abdominal wall. Five months and five days after the operation the cat was etherized, and the anterior wall of the aorta was closely dissected. The pulsations were normal. At the level of the graft there was no dilatation, but the color was red, for the patch had remained a little transparent; then the abdominal aorta was resected. The limits of the patch became immediately apparent, because the aorta contracted while the peritoneal graft did not. The vessel was cut longitudinally. The intima of the aorta and the internal side of the patch were smooth and glistening.[4]

Histological Examination.—The anatomical specimen was cut transversely and the sections stained with hematoxylin and eosin and Weigert's elastic tissue stain. On the section colored by Weigert's method, the limits of the aortic and peritoneal walls are seen very distinctly, because the elastica of the aorta is very dark, while the patch contains very few elastic fibers. These elastic fibers are very thin and are located along the internal side of the wall. The patch is a little shorter than half the circumference of the aorta. Its ends are accurately united to the vessels (Fig. 1). The hematoxylin-eosin section shows that the peritoneal part of the aorta is as thick as the arterial part. Its appearance is almost the same. The aortic wall is normal. The peritoneal wall may be considered as composed of three parts: adventitia, media, and interna. The adventitia contains several vasa vasorum, and is similar to the aortic adventitia. In the media, no elastic fibers are seen. Its internal part is composed of flat connective tissue cells which assume the appearance of muscular fibers; its middle part is composed of irregular connective tissue cells, and its external part of wavy connective tissue fibers. The intima consists of the more internal part of the media. It is characterized by the presence of a number of very thin elastic fibers.

In the third experiment I transplanted on the aorta of a dog a flap of peritoneum together with a piece of the transverse muscle.

Medium sized, young, white bitch. January 31, 1907, under ether anesthesia, semicircular laparotomy and extirpation of a rectangular piece of peritoneum, subperitoneal connective tissue, and transverse muscle. Resection of a piece of aorta about 12 mm. in length and one-third of the aortic circumference in width. The patch is then sutured to the aortic opening. Suture of the muscle to the connective tissue sheath of the aorta. Reëstablishment of the circulation. No hemorrhage. The transplanted flap is thicker than the arterial wall, and bulges above it.

The animal recovers quickly. Pulsations of the femoral remain normal.

November 24, 1908. Etherization. Opening of the abdomen. Pulsations of the abdominal aorta normal. Lumbar peritoneum normal. Dissection of the aorta which is slightly adherent to the vena cava. No evidence of the graft is seen. However, after a close dissection of the anterior wall of the aorta, an elliptical area is seen, the color of which differs slightly from the normal.

[4] Carrel and Guthrie, *Compt. rend. Soc. de biol.*, 1906, i, 1009.

The dimensions of this area cannot be determined, because its limits are not distinct. At its level the caliber of the aorta is absolutely normal. There is not the slightest difference in consistency and thickness of the wall. The circulation is interrupted and the aorta opened. As soon as the vessel is empty of its blood the limits of the patch become immediately apparent because the aorta retracts itself, while the peritoneum does not.

The segment of aorta, on which it is grafted is extirpated, and the circulation is reëstablished by a segment of vena cava.

Œdema of the hind legs for a few days. Pulsations of the femoral arteries remain normal.

October, 1909. Animal in normal condition.

Macroscopical Examination of the Specimen.—On the anterior wall of the resected aortic segment, the peritoneal patch is distinctly seen. Its length is about two centimeters, its width half the circumference of the aorta. Therefore, it is larger than at the time of the transplantation, tweny-two months ago. Its shape is elliptical. On account of the contraction of the aorta it is bent outward. It is a mere cadaveric appearance. Its color, consistency, and thickness appear to be identical with that of the aortic wall. The internal face of the vessel is everywhere smooth and glistening. There is no apparent scar along the line of anastomosis.

Histological Examination.—The vessel is cut transversely at several different levels of the patch. The sections are stained by hematoxylin and eosin, Van Gieson's method and Weigert's elastic tissue stain.

The sections stained by Weigert's method show very accurately the limits of the aorta and of the peritoneal patch. The elastic framework of the aorta is normal and stops abruptly at the point of union with the peritoneum. The union is excellent, the edge of the aorta being slightly bent outward. The peritoneal patch occupies about half the circumference of the aorta. It is almost exactly as thick as the aortic wall. On its internal side a few fine elastic fibers have developed. In the adventitia there is a layer of thick elastic fibers; in the internal part of the media a very large number of exceedingly fine elastic fibers are seen; there is no elastic tissue at all in the external part of the media.

On the sections colored with hematoxylin and eosin the aortic and peritoneal parts of the aorta are hardly discernible. The aorta seems normal, for the peritoneal patch assumes an appearance similar to the aorta (Fig. 2). On both aorta and peritoneum the intima has disappeared. The peritoneal patch is composed of a media and an adventitia. The adventitia contains several vasa vasorum and is identical with the aortic adventitia. The media has the same thickness and almost the same appearance as the aortic media. It is composed of two parts—internal and external. The external part is made of wavy and regular elastic fibers. The internal part consists of regular strata of elongated cells with sacculated nuclei, which look like muscular cells.

The Van Gieson stain shows that they are connective tissue cells. The union between the peritoneum and the aorta is perfect. The muscular fibers are bending themselves outward. At the internal side of the intima elastica elongated connective tissue cells appear; their strata become more numerous and finally substitute themselves for the muscle fiber strata of the aortic wall. There is no scar tissue between the aorta and the peritoneum.

The results of these experiments must be considered from two different standpoints. From a surgical standpoint it is shown that the circulation through the aorta was not modified at all by the substitution of a peritoneal patch for a part of its anterior wall. The operation is not dangerous. The aorta remains normal. There was no danger of embolus because there was no clot on the sutures. It is indicated by the smooth appearance of the line of union of the peritoneum and of the artery. When a small thrombus develops on the sutures the line of union assumes afterwards a scarlike appearance. The results are definitive. Five months after the operation in the first experiment, and twenty-two months after the operation in the second experiment, the animals were opened and the aorta examined directly. The circulation was excellent and the patched aorta was apparently normal.

From an anatomical standpoint the peritoneal patch has adapted itself perfectly to the artery. It has become morphologically identical with the wall of the functionating aorta. When the vessel was examined during the life of the animal it was found that the caliber, consistency and shape of the aorta, together with the thickness of its wall at the level of the patch, were entirely normal. There was only a slight difference in color due, in one case, to the fact that the peritoneum had remained transparent and that, through it, the circulating blood was seen. But, as soon as the circulation was interrupted, the aorta underwent contraction, while the patch did not. Therefore, its limits became immediately very apparent, and it seemed dilated. This apparent dilatation was a cadaveric phenomenon, which appeared when the aorta ceased its function and when the blood pressure was suppressed.

It is very interesting to notice that from a histological standpoint the morphology of the patch has been deeply modified and that its anatomical elements, although unchanged in nature, assumed almost the same appearance as those of the aorta (Fig. 2).

The new wall was composed only of connective tissue. The fibers of the transverse muscle had been absorbed and no smooth muscle fibers had developed. Nevertheless the media looked almost like the aortic media. In the last experiment the internal part of the media was composed of regular strata of connective tissue cells,

the appearance of which was identical with that of muscle fibers. It is a case of cellular mimicry. The elastica of the patch differed deeply from the aortic elastica. In both cases the patch contained a very small amount of elastic tissue. In the first experiment after five months there were only a few elastic fibers in the intima of the patch. No elastic fibers were seen in the media and the adventitia. In the second case, twenty-two months after the operation, there were no elastic membranes to be compared with those of the normal aorta. Nevertheless, a diffuse infiltration of the internal part of the media by exceedingly thin elastic fibers was seen. A few larger fibers had also appeared in the adventitia.

The new wall, therefore, differs deeply from the normal one, in spite of its morphological similarity. It does not contain any muscular fibers. The elastic tissue has taken very little development. Nevertheless, the tissues of which the wall is composed, although not thicker than those of the artery, were able to stand the blood pressure without undergoing any dilatation.

CONCLUSIONS.

These experiments show that an artery can regenerate itself by using heterogeneous anatomical elements. The adaptation of the peritoneal patch to the aortic wall was so perfect that the vessel, less than two years after the operation, was absolutely normal. Although the new wall was composed of tissues different from normal, the morphology of the aorta was not modified. By a kind of mimicry the connective tissue cells assumed the appearance of muscular fibers. The mechanism of this perfect morphological redintegration is as unknown as the cause of the persistency of the form, in spite of the ceaseless mutations of living matter. The power of redintegration of an artery renders possible its patching with tissues, which have by themselves very little resistance. It explains why an aorta patched with a piece of peritoneum can functionate normally twenty-two months after the operation.

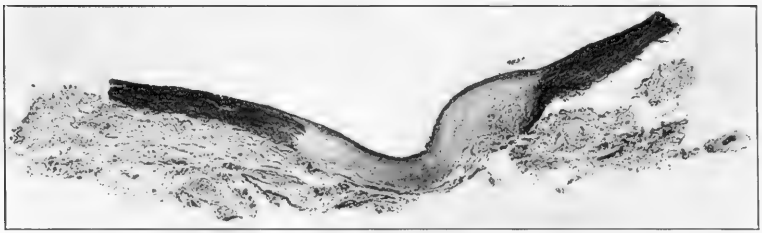

FIG. 1.

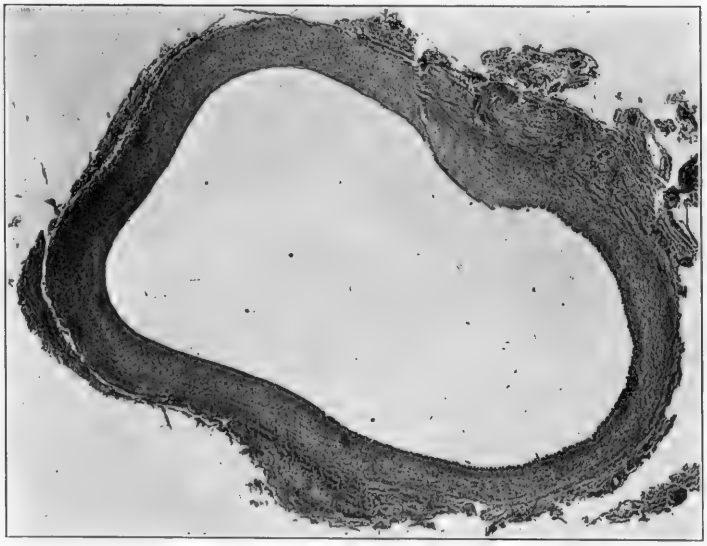

FIG. 2.

EXPLANATION OF PLATE V.

FIG. 1. Peritoneal patching of the aorta of a cat five months after the operation. The elastic framework of the aorta stops abruptly at the edges of the peritoneal patch.

FIG. 2. Peritoneal patching of the aorta of a bitch twenty-two months after the operation.

REMOTE RESULTS OF THE REPLANTATION OF THE KIDNEY AND THE SPLEEN.[1]

By ALEXIS CARREL.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

The technique of the transplantation of organs has been very much improved and simplified in the course of the experiments made during the last two years at the Rockefeller Institute. The grafting of a kidney or a spleen on the renal or the splenic vessels has become an easy and safe operation. Vascular or ureteral complications occur very exceptionally, and, after the operation, the animals remain generally in good health. From a surgical standpoint the problem of the graft of organs can be considered as having been solved.

But, from a biological standpoint, no conclusion has thus far been reached because the interactions of the host and of its new organ are still practically unknown. The study of these interactions was very difficult because the complications which followed the operation were of widely different kinds.[2] In most cases it could not be ascertained whether the cause of the accidents was biological or surgical, and biological causes were sometimes held responsible for accidents due merely to a fault of technique. Therefore, it was necessary to develop a method making it possible to determine the relative importance of the biological and surgical factors in the evolution of transplanted organs.

This method consisted merely in using a technique the details of which were identical whether the organ was grafted on the same animal or on another animal of the same species. Should an organ, extirpated from an animal and replanted into its owner by a certain technique, continue to functionate normally and should it cease to functionate when transplanted into another animal by

[1] Received for publication December 29, 1909.

[2] Carrel, *Jour. of Exper. Med.*, 1908, x, 98.

the same technique, the physiological disturbance could not be considered as brought about by the surgical factors. The changes undergone by the organ would be due to the influence of the host, that is, to biological factors.

The experiments were divided into two series. In the first series, made in 1908, the kidneys[3] or the spleen from a dog were extirpated on the same animal. The experiments of the second series were performed seven months ago. They consisted in extirpating a kidney from a dog and transplanting it into another dog by the same technique as that used in the first series. The present article deals only with the first series of operations, while the experiments of the second series will be published later.

Two points had to be elucidated by the first series of experiments. (1) Can an organ which underwent section of its vessels and of its nerves, a complete interruption of its circulation, and a perfusion with Locke's solution, functionate normally for a very long period of time? (2) What is, then, its anatomical condition?

To the first question is given a positive answer by the following experiment. On February 6, 1908, the left kidney of a bitch was extirpated, washed in and perfused with Locke's solution and replanted.[4] The circulation was reëstablished after having been interrupted for fifty minutes. Fifteen days afterwards the right kidney was removed. The animal remained in perfect health.[5] In June, 1909 this bitch was pregnant and gave birth to eleven pups. In December she again had three pups. To-day, twenty-three months have elapsed since the operation and she is still entirely healthy. Therefore, it is certain that the operation in itself does not interfere at all with the functions of the organ for almost two years at least.

Four other experiments have been performed in order to study the second, point that is, the anatomical condition of the organ.

Experiment I.—Extirpation and replantation of the left kidney. Ether Anesthesia. Middle-aged Irish terrier. November 22, 1908, extirpation of the left kidney, which is put into a jar of Locke's solution and perfused. The kidney is

[3] Carrel, *Arch. f. klin. Chir.*, 1909, lxxxviii, 372.

[4] All the operations were made under ether anesthesia.

[5] The first part of this observation has been published in the *Arch. f. klin. Chir., loc. cit.*

replaced into the peritoneal cavity. Suture of the renal artery and vein. Reëstablishment of the circulation after an interruption of about fifty minutes. Suture of the ureter and of the peritoneum.

The animal remains in good health and is sent to the farm.

September, 1909. Animal becomes sick.

October 14, 1909. Death.

Autopsy.—Very large dilation of the stomach. Stenosis of the pylorus by fibrous adhesion to the liver. Both kidneys are normal. The left kidney does not show any evidence of having been extirpated and replanted. It presents exactly the same appearance as the right kidney. The renal vessels are carefully dissected. The perivascular connective tissue does not present any abnormality. The caliber of the vessels is normal and the location of the anastomoses cannot be detected. The anterior wall of the vein is cut longitudinally and the linear scar of the anastomosis is easily seen. On longitudinal opening of the artery, no evidence of anastomosis is found. Nevertheless, by careful examination, the point of union can be located by a very slight difference of color in a point of the intima. The scar has become practically invisible. The ureteral anastomosis also is perfect.

Experiment II.—Extirpation and replantation of the left kidney. Ether anesthesia. Large young black dog. February 1, 1909, extirpation of the left kidney, which is perfused with Locke's solution. Then the kidney is put back into the abdomen. Suture of the vessels, the ureters and the peritoneum. Reëstablishment of the circulation after an interruption of forty-six minutes.

The dog remains in excellent health.

April 21, 1909, the dog is killed in a fight with other dogs.

Autopsy, eight hours after death. The kidneys are normal. There is no evidence of the operation. Nevertheless the location of the anastomoses is detected by a slight adhesion of the vein to the artery. Longitudinal opening of the vessels: linear scars and no modification of caliber. From both standpoints, microscopical and histological, there is no difference between the left and the right kidney. They are entirely normal.

Experiment III.—Extirpation and replantation of the spleen. Ether anesthesia. Medium sized white and black dog. February 4, 1908, ligature and section of the gastro-splenic vessels, which are abnormally large; ligature and section of the omentum. Dissection and section of the splenic artery and vein which are abnormally small. The spleen is removed and deposited in a jar of Locke's solution.

Replantation of the spleen and suture of the vessels. The lumen of the artery is only one-half millimeter wide, and the suture is difficult. Nevertheless, the circulation is reëstablished normally. The spleen is very red and its volume is increased.

February 18, 1908. Dog in normal condition. Exploratory laparotomy. Adhesion of the omentum to the spleen. Incision of the spleen. Hemorrhage of dark blood. Probable occlusion of the artery. May, 1909, exploratory laparotomy. The spleen has completely disappeared.

Experiment IV.—Extirpation and replantation of the spleen. Ether anesthesia.

February 24, 1908. Large yellow dog, in poor health. Splenic vessels are normal. Extirpation of the spleen which is washed in and perfused with Locke's solution. The spleen is replaced into the peritoneal cavity, and the vessels and nerves are sutured. Reëstablishment of the circulation after an interruption of forty-four minutes. The spleen becomes immediately very red and contracted, and after a few minutes its size becomes very much larger than normal.

June 21, 1909. The dog is fat and in excellent health. Ether anesthesia. Exploratory laparotomy. No adhesions. Spleen has regained its normal size. Splenic peritoneum is whitish in some places. On dissection of the vessels, no evidence of anastomosis is found.

November 4, 1909. The dog died accidentally at the farm.

Autopsy.—Spleen in the same condition as in June, 1909. The anastomoses of the vessels cannot be located. Longitudinal opening of the artery and vein. No scars are seen. The specimen is placed into a jar of formalin. After a short time two transverse, whitish bands appear on the internal surface of the vessels, and indicate the location of the anastomoses. There is no visible scar on the intima. The whitish lines are due probably to a slight sclerosis of the adventitia.

Histological Examination.—Sclerosis of the capsula. Spleen normal.

In four experiments three positive results were obtained. The disappearance of the spleen in Experiment III was due to the occlusion of the artery, which occurred doubtless a short time after the operation. The method is not responsible for this accident. For it is known that such a small vessel cannot be sutured with many chances of success. In that case the end to end suture ought to have been given up, and the union performed by an aortic patch.

In the three other experiments the anatomical condition of the vessels and of the organs was studied a long time after operation.

The renal artery and vein were dissected and opened two months and twenty-one days after the operation in Experiment II. The location of the anastomoses could be detected by very slight thickening of the perivascular connective tissue and by a slight adhesion of the posterior wall of the renal vein to the artery. On the intima of both vessels was a very narrow, circular scar. It is shown by the result of Experiment I that eleven months after the operation the scars of the anastomoses had become almost invisible. The renal vessels and the perivascular connective tissue were absolutely normal, and the anastomoses could not be located. After opening of the vein a linear scar was seen. But the intima of the artery

did not show any scar at all, and the anastomosis could be located approximately only by a slight difference of color of the wall at one point. The suture of the splenic vessels in Experiment IV has given still better results. On the fresh specimen, and after having been opened, the splenic vessels did not show any evidence of ever having been cut transversally. No scar at all was seen. Under the influence of formalin a slight change in color appeared which permitted the location of the anastomoses. It can be considered as certain that these results of the suture of the renal and splenic vessels are definitive.

The anatomical conditions of the replanted organs, kidney or spleen need not be described, for they are entirely normal.

The five experiments mentioned in this article demonstrate that the section of the vessels of the spleen and kidney of a dog, the section of their nerves, the suspension of their circulation for about fifty minutes, and their extirpation and perfusion with Locke's solution do not modify their functions and their anatomical condition when they are replanted on the same animal. These results have been observed for a long time after the operation and can be considered as definitive.

If some changes in the functions and in the structure of the kidneys occur when grafted with the same technique on another dog, it will be permissible to assume that the lesions produced are due to the influence of the host on its new organ. Six dogs, whose left kidneys were replaced seven months ago by left kidneys extirpated from other dogs, are actually living and in good health. They will be used next year for the study of the interaction of the organ and its new owner, and for determining, in some measure, the hypothetical practical value of the transplantation of organs.

THE PHYSIOLOGY OF THE IMMEDIATE REACTION OF ANAPHYLAXIS IN THE GUINEA-PIG.[1]

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Plates VI–IX.

Introduction.—If a guinea-pig be given a subcutaneous, intraperitoneal, or intravenous injection of normal horse serum in appropriate dose, and then, after an interval of ten days or more, this treatment be repeated, it is found that in the elapsed time the animal has undergone a change of such nature that, while the first injection has been without noticeable harmful effect, the second injection causes a very violent poisoning. This reaction is representative of a large group of similar phenomena which can be elicited in the guinea-pig and to a limited extent in other animals by repeated treatments with many native, non-toxic, soluble proteids. The fact as above stated was first observed by Theobald Smith[2] in 1903, and the first systematic work in extension of it was published by Otto[3] in 1906. This was shortly followed by the valuable publications of Rosenau and Anderson of independent work along similar lines.[4] Since then a very voluminous literature has accumulated in regard to this "reaction of anaphylaxis" as it is called, most of which need not concern us at the moment. The work and opinions of others which bear on the points developed by us will be discussed in the course of the paper.

Even considered superficially, this complex reaction presents several distinct phases for examination. The progress and nature

[1] Received for publication January 4, 1910. The chief results here reported in detail were summarized in a note published in the *Jour. of the American Med. Assn.*, 1909, liii, 458.

[2] See Lewis, P. A., *Jour. of Exper. Med.*, 1908, x, 1.

[3] Otto, R., v. Leuthold Gedenkschrift, Berlin, 1906, i, 155.

[4] Rosenau and Anderson, *Hyg. Lab. Bull.*, 1906, No. 29.

of the changes on the part of the animal from apparent indifference to extreme sensitiveness have been the subject of much experimentation and discussion. The reaction to the second injection may be considered quite independently of the preceding changes as an extremely characteristic intoxication due to some substance or group of substances as yet unidentified. The writers heretofore who have paid most attention to this intoxication *per se* have considered it particularly from the point of view of the pathological anatomist; to a less extent and with little decisive experimentation, the functional disturbances involved have attracted attention. The work of Richet[5] and Biedl and Kraus[6] demands especial mention in this connection. These authors have presented careful studies of the functional changes associated with anaphylaxis in the dog. Their results obtained on this animal, as contrasted with our results with guinea-pigs, show that the reaction in the two animals, guinea-pig and dog, is superficially dissimilar, and only further work can show where lie the fundamental similarities which probably exist. In regard to the finer anatomical changes found, widespread fatty degeneration, endotheliolysis, and hemorrhage, it may be said at once that they are consecutive to functional changes and that there is little which decisively distinguishes them from the consequences of the action of a number of other intoxicating agents.

The experiments which we wish to record in the following pages were made in the hope of gaining more definite information respecting the functional manifestations of the reaction of anaphylaxis in the guinea-pig. Moreover, the reaction approached from this point of view is still complex. The results obtained when hypersensitive guinea-pigs are given the second or intoxicating injection of specific proteid vary remarkably, the variations depending on several unstable factors which must be briefly reviewed.

There is expressed in the resulting reaction a relationship at least roughly reciprocal between the sensitiveness of the animal and the amount and injection site of the specific proteid. The subcutaneous injection is least active, the intraperitoneal more so,

[5] Portier and Richet, *Comp. rend. Soc. de biol.*, 1902, liv, 170.
[6] Biedl and Kraus, *Wiener klin. Woch.*, 1909, xxii, 363.

and the intravenous injection perhaps most effective. A highly sensitive animal injected intravenously with a dose of maximum efficiency dies in from two to five minutes, showing a definite and characteristic chain of symptoms, largely respiratory, which will be described in great detail in the following pages. This form of reaction has been variously termed acute anaphylaxis or anaphylactic shock. The term "shock" having already a well defined meaning in physiological literature, and the word "acute" applied to any condition implying that there is a definitely known chronic condition due to the same cause, it seems less confusing to call this fulminating reaction the "immediate" reaction of anaphylaxis.

If the intoxicating dose be given subcutaneously in animals ordinarily hypersensitive, the course of the intoxication is prolonged and its symptoms are quite different. In a most extreme example, the animal becomes sick in about an hour after the injection, and dies in from four to six hours, or after the sixth to the twelfth hour shows distinct and finally rapid recovery. In this type of reaction the respiratory convulsions may be quite lacking. The animal's coat becomes rough, he is cold, and sleepy. He shivers, lies down frequently and stretches full length. In the interval he gets up, huddles in the straw, or with his fellows in the corner of the cage. The respiration becomes more and more feeble and finally disappears. The anatomical changes, fatty degeneration and hemorrhage are usually extreme in this type of case.

The average result of a subcutaneous intoxicating injection of the proteid, the usual result of intraperitoneal injection, and a very unusual consequence of the intravenous administration of a certainly fatal dose, is a complex of this slow type of reaction and the convulsive immediate reaction which we are particularly concerned with in this paper. We wish to emphasize duly the point that our results bear only upon the immediate reaction following most characteristically upon the intravenous injection of the intoxicant in excess, and that we have no definite data which seem at the present writing to account for the slow form of the intoxication just described. Naturally, in the average complex case resulting, let us say, from an intraperitoneal injection in a moder-

ately sensitive animal, our work explains a part of the symptomatology, but not the whole.

Experiments.—All of our experiments were carried out with guinea-pigs, for in these animals the symptoms of immediate anaphylaxis may be produced with great ease and comparative certainty. Sensitization was caused by the subcutaneous injection of either one to five cubic centimeters of horse serum or by three to four milligrams of edestin dissolved in N/20 sodium hydrate, so as to form a one per cent. solution. The lethal dose was almost invariably injected intravenously, usually through a cannula, into the jugular vein; this dose varied, with horse serum, from 0.1 cubic centimeter to one cubic centimeter, and with edestin, from 0.5 milligram and less to ten milligrams. The interval between the sensitizing and toxic doses varied from three weeks to over six months.

Symptoms of Immediate Anaphylaxis.—The picture presented by immediate anaphylaxis in guinea-pigs varies somewhat with the position of the animal. If the sensitized animal is at once released from the holder after an intravenous or intracardiac injection of horse serum or edestin, it at first remains quiet, but within one minute restlessness develops; the hair begins to bristle over the neck and head; it sits up on its hind legs and vigorously rubs the nose, giving frequently a spasmodic moist ringing sneeze; occasionally there is a jump. Within two minutes violent tonic and clonic convulsions develop, and the animal can no longer stand, but falls on its side; the legs are neither rigid nor flaccid, and a sharp kick is caused by a moderate squeeze of the toes. Now the mouth opens with each respiration, which is very slow and finally ceases usually within three minutes after the intracardiac injection. The heart still beats regularly for many minutes after all respirations have stopped.

If, on the other hand, the animal is kept stretched out on its holder, the train of symptoms is somewhat different, and signs come into evidence which have been largely masked when the animal has been at liberty. To illustrate this difference the toxic dose of serum or edestin has been injected into the circulation after the animal has recovered from the ether anesthesia used for intro-

ducing a cannula into the external jugular vein. In less than one minute the respirations quicken and the animal becomes restless and squeaks shrilly. Now it can be noticed that the sides of the chest sink in moderately with each inspiration. This sinking in of the chest with each inspiration becomes rapidly more pronounced, until finally the sides and lower portion of the sternum are depressed very strongly with each inspiratory effort. In this stage the respirations are slow, labored, and the animal shortly shows tonic and clonic convulsions, but gives vent to no sound, or at most to a choking squeak. At the same time, the pupils dilate. The mucous membranes of the mouth and the tongue become bluish, and often a spurt of urine is seen. The convulsions now cease and the animal lies motionless; it is not yet dead, for the heart is always seen beating with strong, regular, but slow pulses. After a respiratory stoppage which usually lasts one minute, respirations again appear, this time accompanied by opening of the mouth. These respirations are slow and cause apparently but little movement of the chest; they gradually become weaker and in another minute or two only the mouth is seen opening slightly a few times. Now all respiratory attempts have disappeared permanently, but the heart is still beating regularly, though usually more slowly and weaker than during the respiratory stoppage which just precedes the last attempts of the animal to breathe. These heart beats may still be observed many minutes after all respiration has stopped. The entire process from injection of the lethal dose to the permanent cessation of respiration lasts less than five minutes.

From the two descriptions of immediate anaphylaxis given above, it will be seen that the two methods of observation lay stress on two different sets of organs. When the animal is loose the paralytic symptoms of the skeletal motor mechanism dominate the picture; when the animal is fastened on its back on a suitable holder, marked respiratory symptoms force themselves upon the observer, which are largely masked in the first method of investigation. On account of the prominence of these changes in the respiration we investigated this function carefully during the anaphylactic state. Before entering upon this, however, we will briefly mention the autopsy findings in a typical case of anaphylaxis in the guinea-pig.

Autopsy Picture.—The peritoneal cavity usually offers but little of interest. The gut is usually found moderately congested, especially the beginning of the colon. The small intestine may show active, coördinate peristalsis, which at times is quite marked and almost resembles Van Braam Houkgeest's "Rollbewegungen."[7] These last but a short time, when present. No definite peristalsis of the colon, cecum or stomach were seen. The ureters were usually congested and responded readily with a peristaltic contraction to a touch. As a rule no hemorrhages were seen in the stomach or colon of guinea-pigs which died within five minutes after injection of the toxic dose. The diaphragm often shows hemorrhages and is much less arched than in normal animals after death. On opening the chest the lungs present a striking sight; the lungs do not collapse, as normal lungs do when the thoracic cavity is opened, but remain almost fully distended. They look pale bluish pink, and apparently form a cast of the thoracic cavity. Even when excised *in toto* there is practically no collapse and the posterior surfaces often clearly show the markings of the ribs. The lungs are light, soft and spongy and float on water like a cork. On cutting away pieces of lung tissue these pieces do not collapse, but remain distended; the cut surface is usually dry and on pressure a good amount of air may be expressed. Occasionally this pressure reveals some small foci of white foam, as if there were beginning pulmonary edema; occasionally small hemorrhages were seen on the surface of the lungs. The trachea and bronchi usually were dry, but showed often a marked congestion of the mucosa. The blood in the lungs and in the aorta was black; the heart was usually still beating vigorously when the autopsy was performed; incision in the aorta, abdominal or thoracic, showed spurts of black blood. The heart muscle often showed hemorrhages, which in some instances were very extensive.

From the details which we have given above it will be seen that we are largely in agreement with the anatomical findings given by other writers on the subject, especially Gay and Southard.[8] We differ, however, in one particular, which we think vital, and that is the significance of the condition of the lung in immediate anaphylaxis. None of the previous investigators, as far as we are aware, have attached sufficient importance to this organ; nor has its appearance been accurately described; the striking fact that the lung in acute anaphylaxis seems to remain in an inspiratory, distended condition with open thorax, with unobstructed trachea and large bronchi, and without obvious pulmonary edema, has been noted, as far as we are aware, only by Gay and Southard.[8] These authors call attention to the lungs, describing the condition as an emphysema.

[7] Houkgeest, *Arch. f. d. ges. Physiol.*, 1872, vi, 266; described also as "Peristaltic Rush" by Meltzer and Auer, *American Jour. of Physiol.*, 1907, xx, 259.

[8] Gay and Southard, *Jour. of Med. Research*, 1908, xix, 22–24.

They are under the impression that this condition is produced by an extrapulmonary mechanism in which the diaphragm is an important factor. The significant fact that the lungs on removal from the body do not collapse does not seem to have impressed them particularly.

This immobilization of the lungs we consider the most characteristic sign of immediate anaphylaxis in the guinea-pig, and, indeed, we use it as an indicator of the immediate anaphylactic state in conditions when the animal is motionless, as when under the influence of curarin or when pithed. In the course of this paper we will bring forward evidence that this condition of the lungs forms the anatomical basis for the explanation of immediate anaphylactic death in guinea-pigs.

Respiratory System.—The respiratory changes during immediate anaphylaxis were investigated by various methods. Sometimes the animal was allowed to breathe from a bottle of four to six liters capacity, care being taken that the tubing between bottle and trachea was as short and as wide as possible. Registration was secured by connecting a Marey tambour with this bottle. The air capacity of this *Vorlage* sufficed for the needs of a guinea-pig during five minutes; if a longer period was necessary the bottle was quickly ventilated. Another method of recording respiration was frequently employed, often in conjunction with the previous one; a Meltzer pleural cannula[9] was inserted in a pleural cavity and the intrathoracic pressure changes were recorded by a Marey tambour. Negative pressure in the opened pleural cavity was only partially reëstablished in order to prevent the lung from plugging the opening of the pleural cannula. This method recorded the volume changes of the pleural cavity. Under special conditions (medulla and cord destroyed or curarin), the volume changes of the lung under artificial respiration were sometimes recorded by placing the animal up to the neck into an air plethysmograph or indicated by means of a receiving tambour fixed directly to the chest.

Examination of the various curves obtained give much information about the functional changes going on in the pulmonary

[9] Meltzer, *Zeit. f. Instrumentenkunde,* 1894; abstract in *Cent. f. Physiol.,* 1896, x, 536.

mechanism during acute anaphylaxis. Fig. 1 (Plate VI) shows the oscillations of intrathoracic pressure which are produced by the action of the respiratory muscles, and the blood pressure curve of an anaphylactic guinea-pig. The respiratory curve (inspiration = downstroke) shows graphically what has already been described, namely, the initial quickening of the respiration after the toxic dose, followed by a swiftly developing slowing of the rate with active expiration, then the respiratory stoppage which may last one to two minutes and which is occasionally broken by single respirations with powerful active expiration; finally, the terminal group of respirations. It will be noticed that all of the respirations, excepting the last few of the terminal group, produce a greater negative pressure in the thorax than the normal respirations. Under ordinary conditions this would mean that a larger quantity of air would enter and leave the lungs in the respirations during anaphylaxis, for normally the lungs follow passively the changes in volume of the thoracic cavity which the action of the rib-muscles and diaphragm produce. In the present instance these changes in intrathoracic pressure do not represent respirations, but only respiratory attempts. This is shown clearly in Fig. 2 (Plate VII). In this experiment the animal breathed the air contained in a large bottle and its respiration was recorded by connecting a tambour with this *Vorlage*. A tracing obtained by this method, therefore, gives a roughly quantitative record of the air which leaves the bottle during an inspiration and of the air which enters during an expiration. This instructive curve shows strikingly that during the stage when the respirations are slowed *less air enters and leaves the lung, not more,* as a casual interpretation of the intrathoracic pressure changes seems to show. Again, Fig. 2 shows beautifully that the air in this stage of slowed respiration *enters and leaves the lung with increasing slowness and difficulty,* for inspiration (downstroke on curve) and expiration (upstroke on curve) take place only gradually, as a glance at the record will show.

Moreover, air enters and leaves the lung slowly in spite of the fact that the negative intrathoracic pressure is greater than normal in these respirations (see Plate VIII, Fig. 3) and this increased negative pressure is established at once and not gradually during a res-

piration, so that conditions seem very favorable for a prompt and speedy entrance of air into the lungs. In the terminal group of respirations *we see that practically no air leaves the bottle during an inspiration,* while the intrathoracic pressure shows changes at first which may be even stronger than those registered when the animal was breathing normally, before injection of the toxic dose. These facts which we have brought forward indicate clearly that some stenosis is gradually produced in the pulmonary passages in immediate anaphylaxis, so that in the final stage practically no air enters or leaves the lung in spite of violent respiratory attempts. Since air enters and leaves the lung with greater and greater difficulty, until finally practically none is obtained, it is clear that an asphyxia must swiftly develop. This is the case. The evidence presented thus far amply demonstrates that this must be true, but there are other facts in support of this which may be mentioned. When a blood pressure tracing is taken it will be noticed that the blood in the cannula swiftly darkens and finally becomes black; again, there are convulsions during this stage (Fig. 3), the tongue becomes bluish, the pupils dilate widely, and the blood pressure usually, though not always, shows a considerable rise (Fig. 1). All these facts find their explanation in the speedy development of an asphyxia caused by a pulmonary stenosis, which is brought on by the injection of the toxic dose. It may, therefore, be considered established that the primary cause of immediate anaphylactic death in guinea-pigs is due to asphyxia; cessation of respiration is secondary to this asphyxia.

Section of Vagi: Curarin.—In numerous experiments the effect of the vagi upon the lungs was studied. At this point we may state that section of both vagi in the neck had no preventive effect upon the production of immediate anaphylactic death, such as one might legitimately expect, if the action of the injected toxic dose were in the medullary centers. These experiments, however, do not form a rigid proof of the peripheral nature of the vital change in the lungs; this will be given later.

In many experiments it was desirable to immobilize the animal. For this purpose a liberal dose of curarin (one or two milligrams) was injected into the circulation and accomplished its effect perfectly without interfering to any noticeable extent with the cardiac vagus. In these curarized animals, also, the effect of the toxic injection was promptly and characteristically shown; arti-

ficial respiration being, of course, given after the curarin. These curarin experiments, it may incidentally be stated, refute the theory of Gay and Southard[10] that immediate anaphylactic death seems to require stimulation of the medullary or phrenic centers, thus producing a diaphragmatic spasm and pulmonary emphysema. In these curare experiments the diaphragm was excluded, the phrenic nerves being physiologically cut, yet the typical and death-bringing lung condition developed with promptness when the second injection was given.

Peripheral or Central Action.—In a preceding section we have demonstrated that asphyxia is the killing agent in immediate anaphylactic death, and that this asphyxia is produced by a stenosis of the pulmonary air passages. How is this stenosis produced? Is it the result of a stimulation in the central nervous system, as is generally assumed, or is it produced by action upon the peripheral mechanism itself in the lung, or do both factors, a central and a peripheral action, join in bringing about this condition? A survey of the recent literature bearing upon this question shows that practically all observers agreed that the main phenomena in acute anaphylaxis were due to some disturbance of the medullary centers. Thus, for example, Richet in a recent paper defines the "poison" which produces all the phenomena of anaphylaxis (apotoxin) as a poison which paralyzes the central nervous system and especially the vasomotor functions of the central nervous system.[11] Rosenau and Anderson[12] suggest repeatedly that the "essential lesion of serum anaphylaxis is probably localized in the respiratory center." Besredka[13] explains the phenomena under discussion as the result of changes occurring in the nerve cells. Gay and Southard speak of a local anaphylaxis of the respiratory nervous center.[14] The only investigators who considered the peripheral nature of anaphylaxis were Biedl and Kraus,[15] who used the dog as the experimental animal. Biedl and Kraus furnished evidence that the characteristic anaphylactic effect in dogs, the prompt drop in blood pressure, was caused by a peripheral paralysis of the vasomotor system.

[10] Gay and Southard, *Jour. of Med. Research,* 1908, xix, 23–26.

[11] Ch. Richet, *Ann. de l'Inst. Pasteur,* 1909, xxiii, 797.

[12] Rosenau and Anderson, *Hyg. Lab. Bull.,* 1908, No. 45, p. 61; also *Hyg. Lab. Bull.* No. 29.

[13] Besredka, *Bull. de l'Inst. Pasteur,* 1908, vi, 948.

[14] Gay and Southard, *Jour. of Med. Research,* 1908, xix, 24.

[15] Biedl and Kraus, *Wiener klin. Woch.,* 1909, xxii, 366.

From this short survey it will be seen that practically all investigators (all of those who experimented with guinea-pigs) were committed to the theory that the vital effects of immediate anaphylaxis were exerted in the central nervous system.

Numerous experiments were made to strengthen this hypothesis, but none were conclusive. Yet technically it was much easier to attempt to demonstrate a possible peripheral action in acute anaphylaxis; all that is necessary is to destroy the central nervous system, keeping the animal alive by means of artificial respiration. If a characteristic reaction is now obtained after injection of the toxic dose, incontestable proof will have been furnished that the central nervous system is negligible in its production; in other words, that the action is peripheral. But, for a successful demonstration by this means, a characteristic reaction, if possible the vital reaction, must be known, and we think that we have this vital reaction, as far as immediate anaphylaxis in guinea-pigs is concerned. We have already stated, and advanced the proof, that the most characteristic, the vital reaction of immediate anaphylaxis in guinea-pigs is the production of a stenosis in the pulmonary air passages, so that in the final stages very little or no air enters or leaves the lungs in spite of violent respiratory attempts, *and that anatomically this condition is easily recognized.* This striking behavior of the lung we have used as an indicator that immediate anaphylaxis had developed as a result of the toxic dose. The course, then, of an experiment to demonstrate the peripheral action of the toxic dose was as follows: Sensitized guinea-pigs were anesthetized with ether, suitable provision being made to maintain the animal's temperature (electric pad); a cannula was tied into the trachea; both vagi were cut in the neck, and artificial respiration was started. Now the animal was turned on its belly, and the cord, medulla, and the lower portions of the brain were destroyed by pithing. Then the animal was returned to its original position, on its back, and a cannula tied into the external jugular vein. Great care was exercised that the artificial respiration (40 to 60 per minute, occasionally more) was sufficiently strong to give a well defined chest expansion to the animal. Now after determining that the heart was beating, the toxic dose was injected into the cannula, the cannula being

washed clear by one cubic centimeter of normal salt or Ringer's solution. Careful observation showed that within one to three minutes after the injection, the chest expansion gradually grew less, the chest becoming fuller, until finally the blast of air driven rhythmically into the trachea caused no further expansion or collapse of the chest. The chest was fixed motionless in a more or less inspiratory condition. Fig. 4 (Plate IX) illustrates this well. This tracing was obtained from a pithed animal by means of a tambour fixed over one side of the chest. Each oscillation represents a blast of air from the respiration machine, upstroke being produced by an expansion of the chest. Shortly after the intravenous injection of 0.2 cubic centimeter of normal horse serum it may be noticed that the respiratory oscillations increase slightly in size, then a rather rapid diminution in size of the respirations occurs, until finally practically none are seen, which in this instance is one and a half minutes after injection of the toxic dose. Inspection of the tracing will also show that the quiescent chest now traces a line distinctly higher than the line formed by the bases of respiratory oscillations (the expiratory level) which were obtained before injection of the toxic dose; this means that the chest is now at rest in a more expanded condition than during the period of expiration before injection. Autopsy of this animal showed the typical picture of anaphylaxis of the lung which we described before.

A more striking tracing, and one which usually shows more details, may be obtained, if the pressure changes in the thoracic cavity are recorded by means of a pleural cannula. Fig. 5 (Plate IX) (pithed animal) is a tracing obtained in this way. This curve shows shortly after injection of the horse serum that the oscillations, produced by the effect of artificial respiration upon the volume of the lung, at first become slightly less, then increase in size and finally rapidly decrease in extent, until nothing but the volume changes of the heart are recorded. These changes indicate that the toxic dose first causes a slight decrease in the volume changes of the lung, then an increase in the volume changes, and finally, one and three quarter minutes after the injection, a decrease to such an extent that no volume changes of the lung are recorded at all, although artificial respiration is still continued

exactly as before, the small waves visible being those caused by systole and diastole of the ventricles.

Since we have obtained the characteristic lung picture of immediate anaphylaxis invariably in these pithed animals, of which Figs. 4 and 5 give a graphic presentation, we have furnished absolute proof that the central nervous system is not essential for the production of the characteristic and death-dealing lesion of immediate anaphylaxis; the action is surely peripheral in the lung. But this evidence permits no conclusion regarding a possible effect on the central nervous system; it is quite conceivable that the medulla does aid in the production of immediate anaphylactic death, but we have demonstrated that this action, if present, is not essential, for not even a delay in the onset of the pulmonary inspiratory immobilization after the toxic dose is to be noted in these pithed animals. Rigid proof for this hypothetical central action, which our experiments have proven to be unnecessary for the explanation of acute anaphylactic death, has been furnished by no one.

Causation of Lung Condition.—How is this remarkable condition of the lungs produced? By what mechanism is the air imprisoned in the alveolar sacs, so that the lungs remain distended for a long time when excised? There are several conditions conceivable that would prevent the air from escaping and thus produce this condition: (1) pulmonary edema; (2) emphysema; (3) submucous edema in the bronchi and bronchioli; (4) tetanic contraction of the musculature of the bronchioles, occluding their lumen.

The first possibility, pulmonary edema, may be dismissed at once. The two pictures, pulmonary edema and the lung in immediate anaphylactic death, have nothing in common. In an outspoken edema of the lung, such as may easily be produced in a guinea-pig by an intravenous injection of a fraction of a cubic centimeter of adrenalin, the lungs are dark, mottled, heavy; pink foam usually exudes from the nares of the intact animal; on section the trachea is usually full of a pinkish foam composed of fine bubbles; section of the lung shows it to be extremely juicy. On the other hand, the lung of a guinea-pig dead from immediate anaphylaxis is a pale bluish-pink in color; very light; the trachea contains no foam, and shows often a marked congestion of the blood-vessels; sections of the lung show a fairly dry surface, and on pressure much air, but little fluid, escapes. At times, it is true, some small foci of a fine white foam may be noted in certain sections on pressure, but these were never ex-

tensive nor widespread. These small areas possibly show a slight degree of pulmonary edema in certain areas, but their extent is too small to explain the entire picture.

The second possibility, emphysema, has been noted in immediate anaphylaxis by Gay and Southard.[16] They are even "inclined to regard this emphysema as the effective cause of death in the quickly fatal cases." In the extensive histological studies which these authors give of the lesions found in various organs after acute anaphylactic death, a detailed consideration of the lungs is not given, an omission all the more striking since they attribute such importance to the lungs; moreover, in no place do they explain how an "emphysematous" lung can cause death within a few minutes. We have already shown before that their conception of how this "emphysema" is produced, is erroneous (p. 160).

While Gay and Southard undoubtedly have seen the typical anaphylactic lungs, and although they justly, though with no published evidence, brought the lung condition into causal relation with acute anaphylactic death, it must be pointed out that these anaphylactic lungs are not emphysematous, at least as that term is ordinarily understood. Only two conditions come into consideration if these anaphylactic lungs are emphysematous: acute vesicular emphysema and acute interlobular emphysema. In the acute vesicular type the surface of the lung is raised into little hillocks formed by the overdistended lobules and the areas between these elevations are compressed and even atelectatic; these hillocks are especially noticeable at the free borders of the lobes. In the lung of acute anaphylaxis nothing of the kind is seen; the surface is smooth and the borders of the lobes are clean cut and sharp; in short, the lung looks like a normal lung which has been fully inflated; the histological picture shows no areas of compression or atelectasis, and the alveoli are only moderately distended. Nor is this condition due to interlobular emphysema; in no instance were the characteristic subpleural blebs of air noted on the surface of the lungs. Since there is no other well defined acute emphysema of the lungs, we think that it must be admitted that the lungs of immediate anaphylaxis are not emphysematous.

The third possibility, submucous edema in the bronchi, and especially the bronchioli, is not so easily disproved as the other two possibilities. It is well known from the labors of v. Pirquet and Schick[17] that serum injections may cause edema of the skin in man, especially if the subject has been sensitized by a previous injection of serum. They make no mention, however, of such a condition in the lungs, nor are we aware of any such observation based on anatomical findings. It is conceivable that the sudden development of submucous edema in the bronchioli could practically occlude their lumen, so that air could neither enter nor escape. But while it is impossible to dismiss this explanation, there are a number of facts which speak against submucous edema as a predominant factor in the production of the anaphylactic lung. Thus, for example, we have stated before that after injection of the toxic dose a period of increased lung expansion occurs shortly before the final and complete

[16] Gay and Southard, *Jour. of Med. Research,* 1908, xix, 22; 32–33. See also Gay and Southard, *ibid.,* 1907, xvi, 163–171.

[17] v. Pirquet und Schick, Die Serumkrankheit, Vienna, 1905.

cessation of lung expansion (Fig. 5). How is this increased expansion of the lung to be explained on basis of this theory? The increased expansion obviously means that more air is driven into the lungs by the blasts from the artificial respiration machine; yet, how can more air enter, if, as the hypothesis demands, a submucous edema is being formed, which must to some degree obstruct the lumen of the finer air passages and thus hinder the entrance of more air? Again, how is this theory to explain the fact that an intravenous injection of atropin causes, in suitable cases, a rather prompt resumption of pulmonary oscillations (pithed or curarized animal)?[18] How does the atropin bring about the disappearance of the submucous edema? Some other opposing facts might be mentioned, and will be referred to later, but it seems sufficiently clear now that submucous pulmonary edema *alone* will not explain all of our experimental facts. It is possible, perhaps we may say probable, that the production of such an edema does take place, but conditions do not yet force us to take this factor into consideration in our attempts to explain the experimental data, and under these conditions it is unnecessary to call upon two mechanisms when one of them is sufficient to explain the results.

There remains for consideration the fourth possible explanation, and this is the mechanism which we consider fully capable of explaining the remarkable lung condition in immediate anaphylaxis, namely, a tetanic contraction of the muscles of the finer bronchioles, so that the air is imprisoned in the alveolar sacs. The anatomical basis for this is clear: the finer bronchioles are practically nothing but muscular tubes;[19] moreover muscle fibers are also present in the alveolar ducts[20] and, according to some anatomists, may even form sphincter-like structures about the alveoli.[21] The contraction of these structures must have a profound effect upon the volume of air passing to and from the alveoli. That this is indeed true has been demonstrated by numbers of observers, especially conclusively by Einthoven[22] and Dixon and Brodie.[23] These investigators have shown that stimulation of the vagi in the neck produces a constriction of the pulmonary passages, so that air enters the lung with difficulty, and that this stenosis was produced by a contraction of

[18] The effect of atropin in immediate anaphylaxis will be considered in a later paper.

[19] Oppel, Lehrbuch d. vergleich. mikrosk. Anatomie d. Wirbeltiere, Jena, 1905, 602–603.

[20] Oppel, *loc. cit.*, p. 653.

[21] Oppel, *loc. cit.*, p. 653.

[22] Einthoven, *Arch. f. d. ges. Physiol.*, 1892, li, 367; see also Beer, *Arch. f. Physiol.*, 1892, Sup., p. 138.

[23] Dixon and Brodie, *Jour. of Physiol.*, 1903, xxix, 97.

the bronchial musculature and not by vascular changes as has been assumed by v. Basch and his pupils.[24]

An examination of our lung data will show, we think, that they are easily explainable on basis of this theory. For example let us consider Fig. 5. This curve was obtained from a pithed guinea-pig which had been sensitized by edestin. In one pleural cavity a cannula was fixed and the volume changes of the lungs, produced by artificial respiration from a machine, recorded by a Marey tambour on a smoked drum. The large oscillations represent these changes, upstroke being inflation, downstroke the elastic recoil of the lungs during the respiratory pause. During the time indicated by the broad white mark, one-half milligram of edestin, dissolved in N/20 sodium hydrate was injected into the external jugular vein; the cannula was then washed out by a second injection of one cubic centimeter saline solution. During and after these injections the tracing shows that the oscillations became slightly but definitely smaller; then they increased to an extent greater than normal. Then another slight decrease in amplitude occurred, which lasted about thirty seconds; now within twenty to thirty seconds the amplitude decreased slightly until the pleural cannula recorded no pulmonary volume changes, but only the volume changes of the heart, in spite of the fact that artificial respiration was still continued. These rhythmic changes are now easily explained; the injected substance caused first a broncho-constriction of moderate degree, this was followed in turn by a well defined broncho-dilatation, a slight broncho-constriction, and finally a broncho-constriction of maximal degree occurred so that artificial respiration no longer caused any lung volume changes. Such rhythmic contractions of the bronchial muscles have been described by Einthoven[25] and by Dixon and Brodie;[26] the latter give a curve illustrating this. During the course of this investigation we also have had opportunity to notice such changes after stimulation of the vagi in guinea-pigs; Fig. 6 (Plate IX) shows this very well.

Fig. 5 shows some other points of interest; it will be noticed that

[24] v. Basch, *Arch. f. d. ges. Physiol.*, 1892, lii, 417; Grossman, *ibid.*, 567.
[25] Einthoven, *loc. cit.*, p. 415.
[26] Dixon and Brodie, *loc. cit.*, p. 154.

the lung comes to rest about midway between passive expiration and the inspiration caused by the blast of air. This means that the lung is fuller than during a passive expiration, and more nearly in an inspiratory condition; in other words, air has been imprisoned in the lungs probably by the same agency which first caused a rhythmic change in the lung volume and which finally occluded the pulmonary air passages. In another tracing, Fig. 4, this final resting point was nearer the inspiratory level of the lung oscillations and more air therefore imprisoned. This latter tracing (Fig. 4) shows a slight broncho-dilatation following shortly upon the injection of the toxic dose of horse serum. Both figures show that the air enters and leaves with increasing difficulty by the decrease in size of the lung oscillations and by the increasing slope of these oscillations. Both of these points are well brought out in Fig. 4. This tracing was obtained from a pithed sensitized animal (cord, medulla and base of brain destroyed) by placing a tambour on one side of the chest and thus recording the chest oscillations produced by the expansion and collapse of the lung under artificial respiration from a machine which delivered air to the animal with a constant rate, strength and volume. The terminal oscillations in this tracing are small and slope much more than the normal oscillations which are almost vertical. This sloping upstroke is due to the gradually increasing resistance which the entering blast of air encounters and which can only slowly and slightly change the lung volume; the sloping downstroke shows the slowness with which the lung discharges the air, and finally no lung volume changes occur at all, although the artificial respiration continues exactly as before; no air enters or leaves the lung.

We have stated before that an increase in size of the lung volume oscillations occurs after injection of the toxic dose. This is true only under a certain condition, namely, when the pulmonary bronchioli are in a state of moderate tonus. In the numerous tracings which we have obtained in our study of the anaphylactic lung, practically only those in which the vagi had previously been stimulated have shown in a noticeable degree this primary increase in lung oscillations after injection of the toxic dose. Such broncho-dilator effects may also be obtained by stimulation of the vagi, as

has been shown by Beer[27] and especially by Dixon and Brodie.[28] The latter observers call attention to the fact that this dilator effect may easily be obtained provided that the bronchial muscles are in a state of tonus; a striking curve illustrates their statement.

This fact that a primary broncho-dilatation after injection of the toxic dose is only markedly shown after an effective vagus stimulation is another strong argument in favor of the theory that the bronchial muscles are affected in immediate anaphylaxis.

Finally, we may add that atropin under certain conditions is able to relax the rigid anaphylactic lung so that it is again able to expand and collapse. Now it has been shown by Beer[29] and by Einthoven[30] that atropin paralyzes the bronchial muscles and, therefore, this fact forms another link in the evidence that the bronchial muscles play an important rôle in immediate anaphylaxis in guinea-pigs.

The evidence which we have presented in the last section may be summarized as follows: After the toxic injection (*a*) the lungs of a sensitized guinea-pig which has been curarized or pithed shows within a few minutes a marked decrease in the lung volume changes caused by artificial respiration; this decrease progresses swiftly and finally no volume changes are recorded at all, although the artificial respiration continues with the same rate, force and volume; (*b*) this tremendous decrease in the lung oscillations is preceded under a certain condition by a stage of increased lung oscillations; (*c*) this stage of increased lung oscillations which precedes the final stage of complete absence of lung oscillations may be obtained with a fair certainty only when the broncho-constrictors have shortly before been excited; (*d*) atropin may again bring about lung oscillations even when injected after the final stage of complete absence of pulmonary movements has been established.

Since all the effects mentioned under the first three heads have been produced by stimulation of the peripheral vagus (Einthoven, Dixon and Brodie) and since it has been shown that blood-vascular

[27] Beer, *Arch. f. Physiol.*, 1892, Sup. pp. 208–212.
[28] Dixon and Brodie, *Jour. of Physiol.*, 1903, xxix, 9, 139; tracing on p. 140.
[29] Beer, *loc. cit.*, p. 150.
[30] Einthoven, *loc. cit.*, p. 428.

changes will not alone account for these lung changes, but that the bronchial muscles are the main factors, and since atropin paralyzes the bronchial muscles, we feel justified in assuming that the second or toxic injection in a sensitized guinea-pig acts upon the bronchial muscles, producing swiftly a tetanic contraction of the finer bronchioles which completely occludes their lumen and thus prevents the entrance and escape of air. We do not care, as yet, to state whether the action of the toxic dose is exerted upon the vagus broncho-motor endings, or upon the muscles directly, or upon both.

Blood Pressure in Immediate Anaphylaxis.—The blood pressure in sensitized guinea-pigs was obtained from the carotid artery by means of a Hürthle spring manometer. Mercury manometers we found unsuitable, because the blood pressure oscillations obtained by vagus stimulation allowed a fair amount of the anti-coagulant to flow into the circulation and this introduced a factor of some importance in such a small animal as the guinea-pig, especially when magnesium sulphate or sodium carbonate solutions were employed to fill the tubing.

As anti-coagulant for the connecting tubing we employed chiefly half saturated sodium sulphate solution or 10 per cent. sodium citrate. Their action was not very successful in the guinea-pig, for clots were annoyingly frequent, especially in our earlier experiments when the animals were largely grain-fed.

The average blood pressure at the beginning of an experiment we found to be 80 millimeters of mercury, and the average pulse rate about 275 beats per minute. The animal weighed from 300 to 600 grams. These results agree quite well with those of Harrington.[31] Harrington states that the average blood pressure in his experiments upon guinea-pigs was 75 millimeters (mercury manometer; sodium carbonate for tubing) and the average heart rate was 200.

The blood pressure of an anaphylactic guinea-pig is shown in Figs. 1 and 2. As soon as the respiration shows an increase in rate (Figs. 1 and 2), which occurs within a few seconds after the injection of the toxic dose, the blood pressure begins to rise. This is due to beginning asphyxia, for although Fig. 1 shows at

[31] Harrington, *American Jour. of Physiol.*, 1898, i, 384.

this time an increased negative pressure in the pleural cavity, Fig. 2 shows at the corresponding point that the animal is already experiencing difficulty in getting air into the lungs, for the oscillations of the respiratory curve from the *Vorlage* are smaller and the slopes of the inspirations and expirations are greater. The pulse rate during this stage may not be altered, and the respiratory oscillations of the blood pressure are indicated. As the respiratory difficulty increases the heart may show some irregularities in beats, probably due to reflex vagus inhibition (Fig. 2); if this reflex inhibition is not marked the blood pressure now reaches its maximum (Fig. 1) and then begins slowly to drop, each respiratory effort, even though no air enters the lung, being clearly shown on the blood pressure curve. This drop continues so that five to eight minutes after the maximum the blood pressure has fallen to 10 to 20 millimeters of mercury.

All the blood pressure curves which we have obtained in sensitized guinea-pigs show the same general features: an initial rise, which varies from 10 to 60 millimeters of mercury, shortly after the injection of the toxic dose, a short maintenance of this level and then a gradual drop to 10 to 20 millimeters of mercury. The entire length of time consumed from the injection of the toxic dose to the point where the 10 to 20 millimeter level is reached, usually is less than ten minutes, provided that the animals are highly sensitized and that the body heat of the animal has been maintained during the experiment.

The course of the blood pressure curve in immediate anaphylaxis of guinea-pigs is strikingly different from that in the dog as described by Biedl and Kraus.[32] These investigators noted a fall in blood pressure which occurs about thirty seconds after the toxic injection and reaches its maximum in about one minute. The blood pressure fall is considerable and may reach the 40 millimeter level and even less. Associated with this fall of blood pressure is a diminution in the size of the pulse waves and an increase in the pulse rate. During this stage of low blood pressure, stimulation of the peripheral splanchnic nerves or an intravenous injection

[32] Biedl and Kraus, *Wiener klin. Woch.*, 1909, xxii, 365. See also *Cent. f. Bakt., Ref. Beil.*, 1909, xliv, 68*.

of adrenalin produces no rise of the pressure level, but an intravenous injection of barium chloride does elicit a powerful increase in blood pressure. From these data the authors justly conclude that this fall in blood pressure in anaphylaxis in dogs is caused by a paralysis of the peripheral vasomotor mechanism.

Since the general picture of immediate anaphylaxis in dogs is very different from that of immediate anaphylaxis in guinea-pigs, we need not enter into a further discussion of Biedl and Kraus's interesting communication.

Heart Block.—Each of the blood pressure curves shows more or less well, on mere inspection, that the pulse rate changes markedly during this drop in blood pressure; the beats are large and slow. A count of these pulse beats shows that their rate stands in a simple ratio to the normal number of beats. Thus, for example, the number of beats observable shortly after a complete stenosis of the pulmonary air passages may be 44 for ten seconds; when the first slowing takes place, which occurs abruptly, the beats number 22 per ten seconds; after another slowing these beats may now number 11 per ten seconds. Fig. 5 shows these changes in the heart rate.

Inspection of the animal's heart, when the chest has been opened, shows beautifully the development of a heart block; at first the ventricles beat regularly after a preceding systole of the auricles; shortly the auricles beat two times to every one beat of the ventricles; and a little later the auricles and ventricles beat in a 3:1 rhythm. All of these changes may occur within one minute after the lung bronchioles have become completely occluded by the injection of the toxic dose; Fig. 5 illustrates this change in ventricular rhythm very well. This tracing was obtained by a pleural cannula from a pithed animal, and thus disturbing reflex vagus inhibition plays no rôle in this curve; the blood pressure curves (Figs. 1 and 2) also show at least one change in ventricular rhythm.

Cardiac Vagus During Immediate Anaphylaxis.—In a moderate number of blood pressure experiments we observed the effect of peripheral vagus stimulation upon the heart. Practically all of these experiments showed that during the blood pressure drop the cardiac vagus gradually lost its irritability, so that finally when the blood pressure was about 20 millimeters, we usually obtained no effect on stimulation of the vagus with any strength of induced current.

Effect of Temperature.—In all the experiments which we have made, care was taken to maintain the animal's temperature by means of an electric pad. The rectal temperature noted, after the animal was on the holder, ranged between 37 and 39° C., and this was maintained throughout the experiment with only moderate fluctuations. This we found necessary because a cooled guinea-pig shows a markedly delayed reaction to the toxic injection of serum, and the symptoms which are produced under this condition are not so striking as when the guinea-pig has a higher temperature.

Blood Coagulation.—In the valuable contribution by Biedl and Kraus[33] attention was called to the striking fact that the blood of an anaphylactic dog does not coagulate for days. This is, however, not true for the guinea-pig, as the same authors state in their second communication,[34] and we are able to corroborate this fact. Most of our blood pressure experiments illustrate this fact.

Controls.—In a number of normal guinea-pigs horse serum was injected into the external jugular vein and its behavior observed. In accordance with the result of numerous previous observers we found that the injected horse serum, which varied in quantity from four to six cubic centimeters, exerted no deleterious effect upon the animal. The animals struggled occasionally in the holder, now and then showed some dyspnea (no tracheotomy was performed), but not any more apparently than normal non-operated animals show when merely stretched out on the holder. The blood pressure (Hürthle) after thirty to fifty minutes usually showed a drop of 10 millimeters of mercury. In short, the injected horse serum exerted no obvious harmful effect.

SUMMARY.

1. By an immediate anaphylactic reaction we mean the chain of symptoms which occur in highly sensitized guinea-pigs shortly after an intravenous or intracardiac injection of the toxic dose and usually end in death.

2. Immediate anaphylactic death occurs three to five minutes after the toxic injection in highly sensitized guinea-pigs.

3. Immediate anaphylactic death in guinea-pigs is caused by asphyxia; cessation of respiration is secondary to this asphyxia.

4. This asphyxia is apparently produced by a tetanic contraction of the smooth muscles of the bronchioles, which occludes their lumen gradually, so that finally no air enters or leaves the lung, in spite of violent respiratory efforts; the animal is strangulated.

5. The stage of complete broncho-constriction is preceded by a short broncho-dilatation, if the bronchioles have been in a state of tonus previous to the injection of the toxic dose.

6. Anatomically, the lungs of these guinea-pigs are typical and

[33] Biedl and Kraus, *loc. cit.*, p. 367.

[34] Biedl and Kraus, *Cent. f. Bakt., Ref. Beil.*, 1909, xliv, 70*.

may be used as an indicator of the immediate anaphylactic state when the animal has been immobilized by curarin or by pithing.

7. The lungs of a guinea-pig killed by immediate anaphylaxis are distended and in an inspiratory position so that the diaphragm is pushed down; no marked collapse occurs when the chest is opened and when the lungs are excised *in toto;* their color is a pale bluish-pink; the surfaces and borders are smooth; no foam is in the trachea or large bronchi; pieces of lung cut off do not collapse, float lightly on water, and contain a good amount of air and little fluid which escapes on pressure. The blood in the lungs and heart is black when the autopsy is made at once after the cessation of respiration.

8. Section of the vagi in the neck, or curarin (artificial respiration) exerts no appreciable effect on the development of immediate anaphylaxis.

9. This immobilization of the lungs, which is due to a broncho-constriction, is of peripheral origin, for destruction of the spinal cord and medulla affects in no appreciable way the promptness and extent of the typical lung response to the injection of the toxic dose. Artificial respiration is, of course, necessary.

At the present time we do not care to state whether the toxic dose exerts its effects upon the bronchial muscles alone or upon the vagus motor endings or upon both structures.

10. The blood pressure in immediate anaphylaxis first shows a rise, which may be considerable; a short maintenance of this high level and then a gradual drop to 10 to 20 millimeters of mercury and even less, within ten minutes after injection of the toxic dose.

11. Shortly after injection of the toxic dose a heart block develops, so that auricles and ventricles may beat in a 3:1 rhythm; the block is probably due to asphyxia.

12. The cardiac vagus gradually loses its irritability after injection of the toxic dose.

13. Cooling of the guinea-pig delays the reaction to the toxic injection.

We wish to express our thanks to Dr. W. H. Park for his kindness in furnishing us horse serum.

EXPLANATION OF PLATES.

PLATE VI.

FIG. 1. In this tracing a simultaneous record of the intrapleural pressure and the blood pressure (Hürthle) was recorded. Time marking is in two second intervals. The unbroken line above the time line is the abscissa for the blood pressure tracing. The curve above this, intersecting the large respiratory oscillations, is the blood pressure curve; the blood pressure scale, showing the value of the curve in millimeters of mercury is at the termination of this curve. The curve showing large oscillations represents the fluctuations of the intra-thoracic pressure due to respiration; downstroke equals inspiration. The longitudinal, unbroken line near the top of the large respiratory waves is the atmospheric pressure line; above this line is positive pressure, below is negative pressure.

At the place marked by crosses, 1 c.c. of horse serum was injected into the external jugular vein; this was followed by 1 c.c. of Ringer's solution to wash out the cannula. The blood pressure curve is marred by a partial clotting, but the main features (see text) are well-brought out. The respiratory curve is self-explanatory.

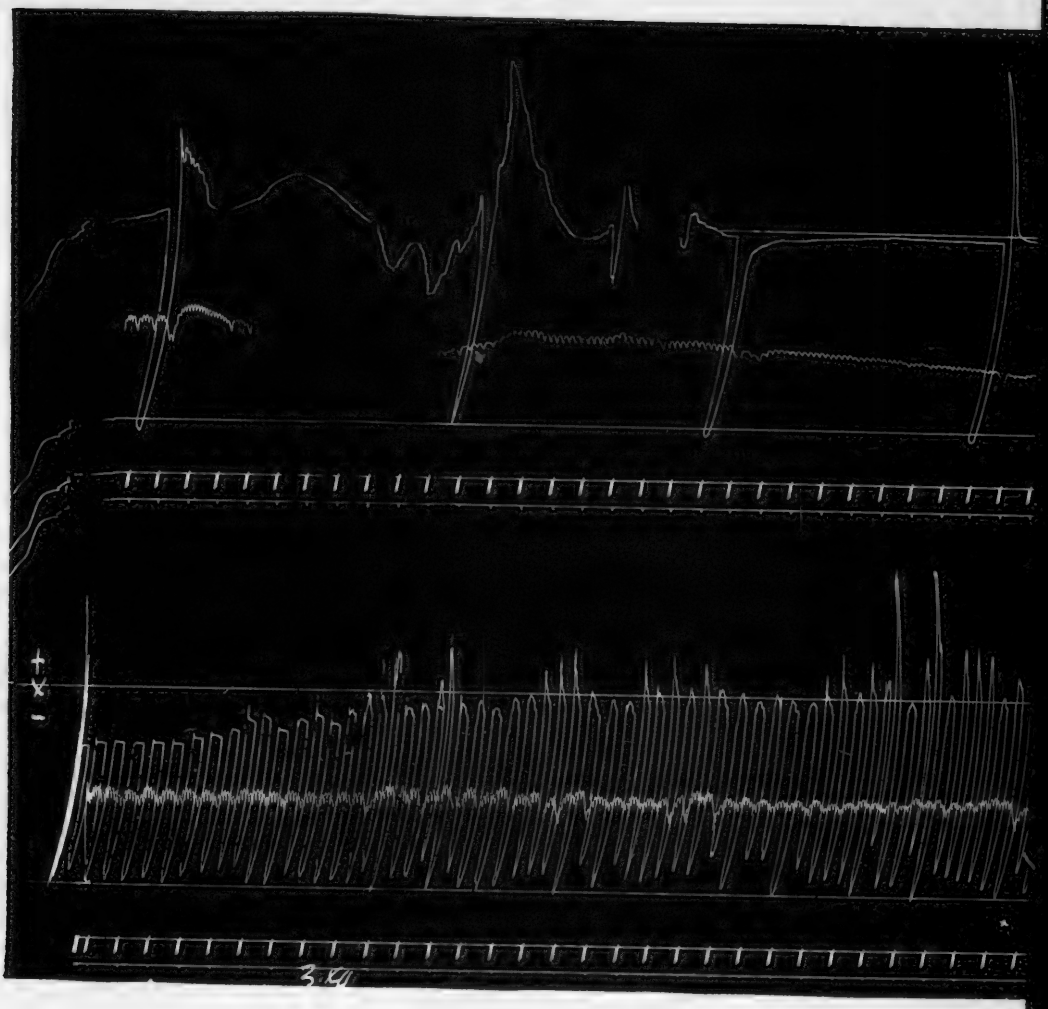
PLATE VII.

FIG. 2. Simultaneous record of the blood pressure (Hürthle) and of the air which enters and leaves the trachea, recorded by means of a tambour connected with the air bottle from which the animal breathed; downstroke equals inspiration. Time is in one second intervals. At one point noted in the curve the air in the bottle was renewed (unnecessarily). The respiratory oscillations immediately following this show very well the increasing difficulty of getting air into and out of the lungs until finally no air enters or leaves, as is shown by the straight line traced by the tambour lever, although the animal makes violent respiratory attempts, as is shown by the respiratory oscillations of the blood pressure curve. The blood pressure shows the same general features as before; note the change in the heart rate.

At the place marked by crosses, 1.25 c.c. of horse serum were injected into the external jugular vein; this was followed by 1 c.c. of Ringer's solution.

PLATE VIII.

FIG. 3. Simultaneous record of intrapleural pressure changes and of the air inspired and expired (*Luft Vorlage*); downstroke on both curves is inspiration. Time marking is in four second intervals. The upper curve, showing large oscillations, records the intrapleural pressure changes; the lower curve indicates the air which enters and leaves the lungs during respiration; the sudden drop at one point of this curve was due to the opening of a stop-cock. The broad white bands on the line beneath the time marking show when 1 c.c. of horse serum and 1 c.c. of saline solution were injected into the external jugular vein. Shortly after the serum injection the respirations became slower and dyspneic; the intrathoracic pressure changes (upper curve) are greater while the lower curve shows that less and less air enters and leaves the lung. Shortly after this no air enters or leaves the lung and the lever of the tambour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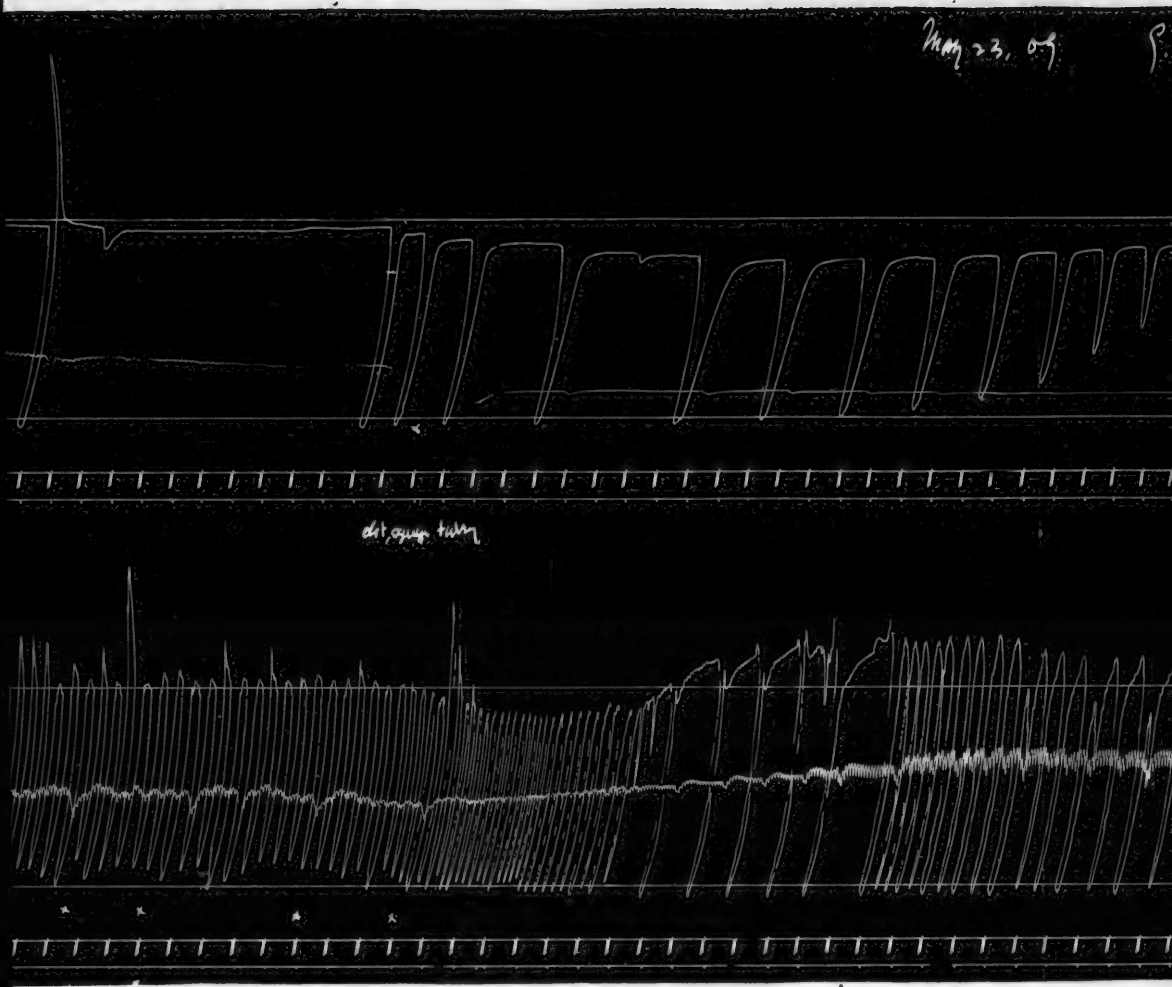


FIG. 1.

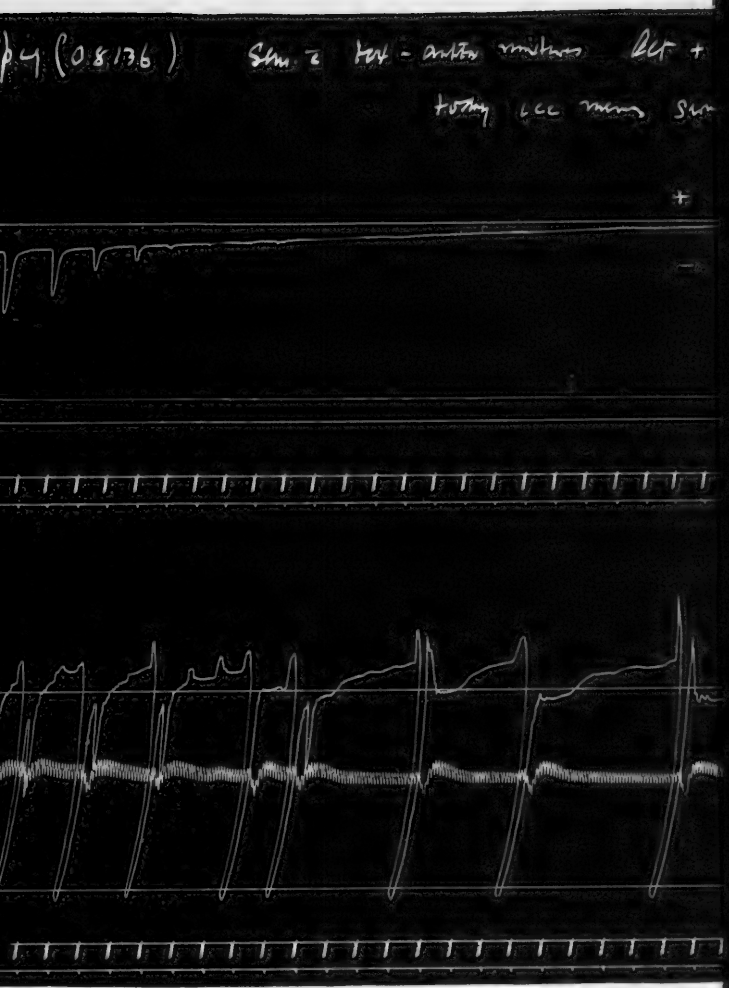
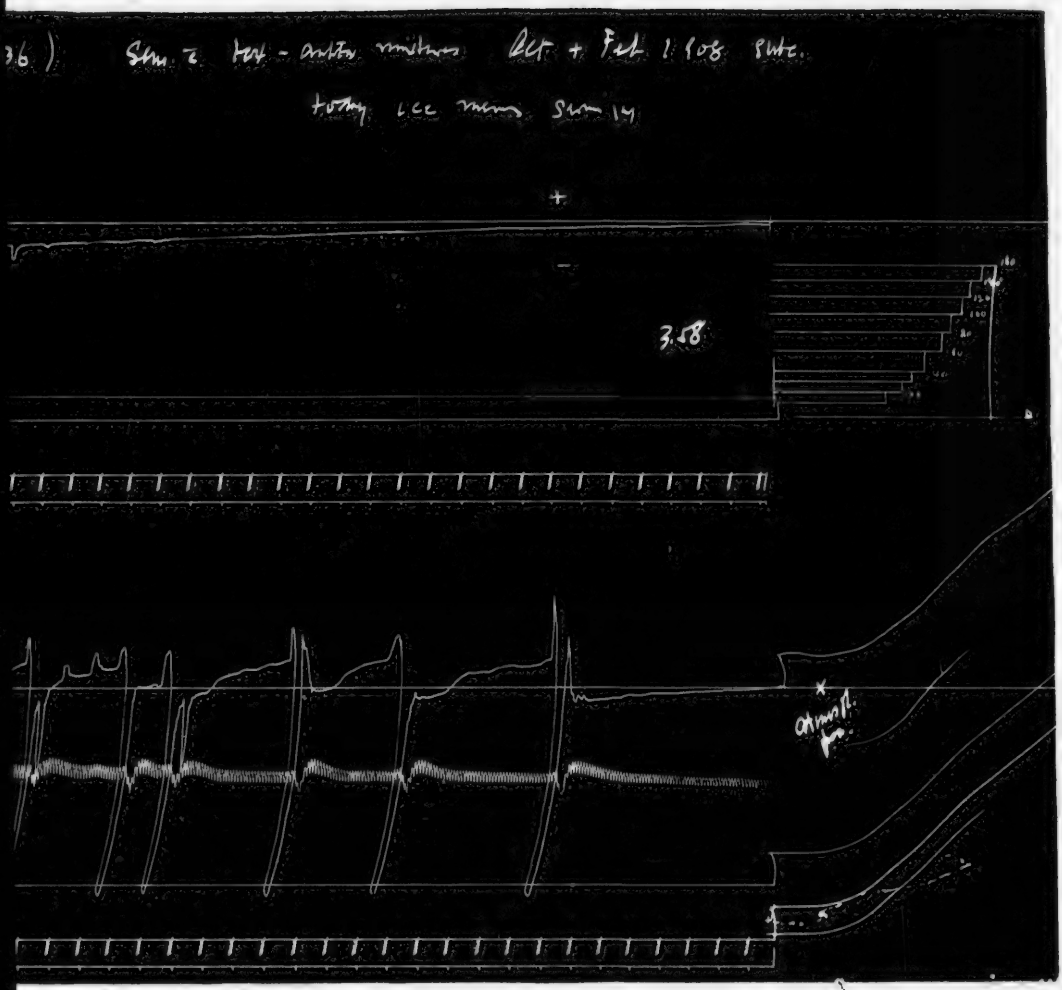
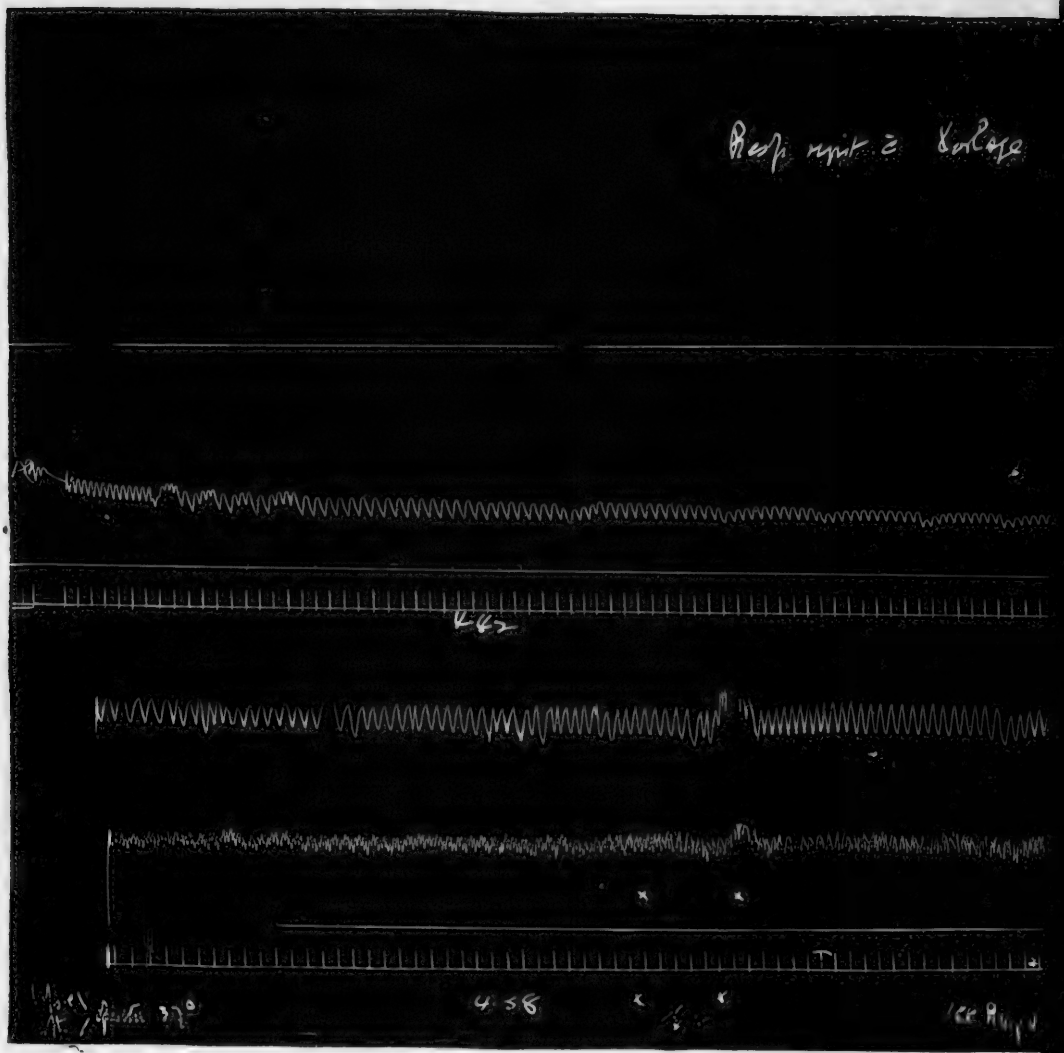


PLATE VI.





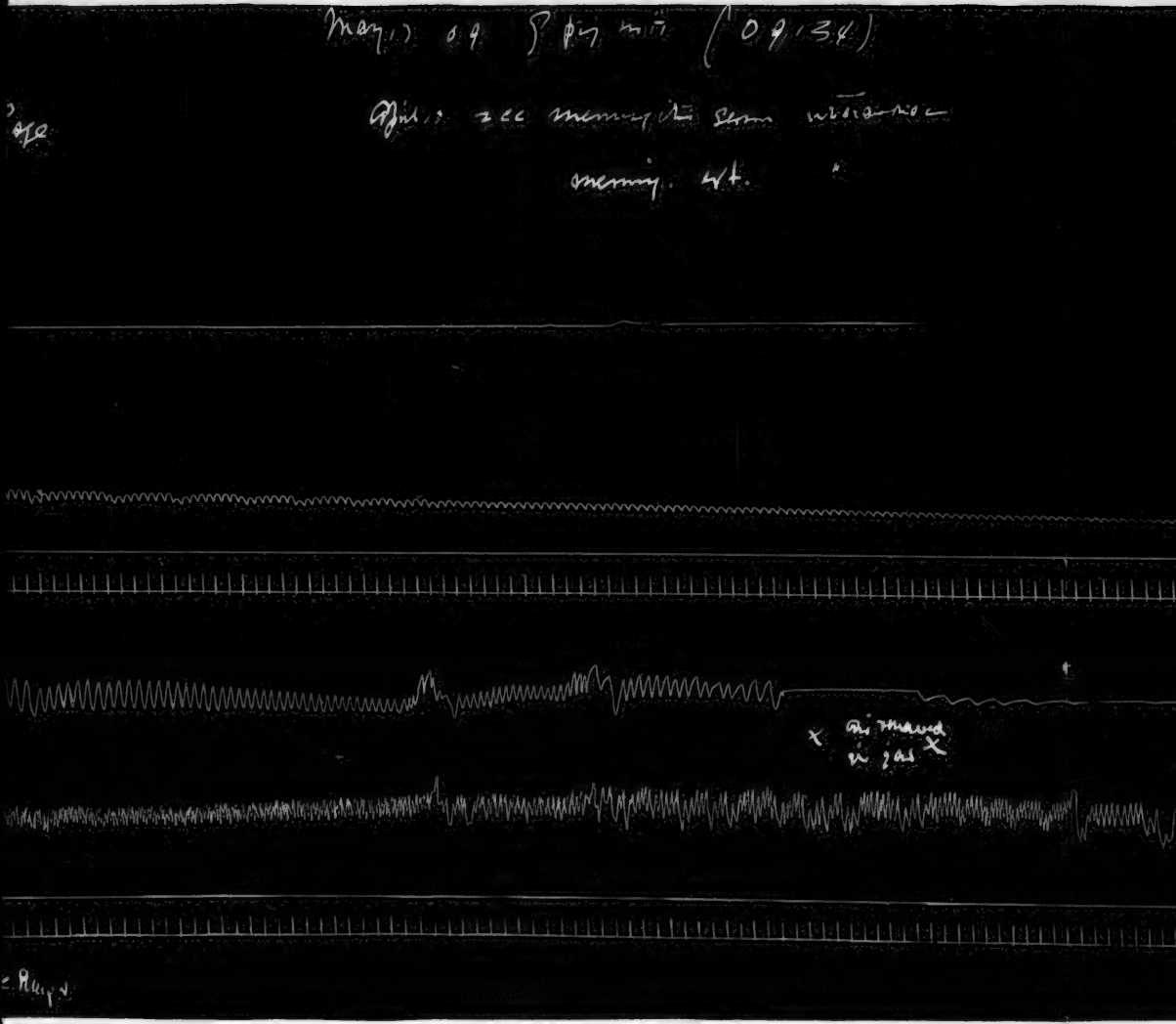
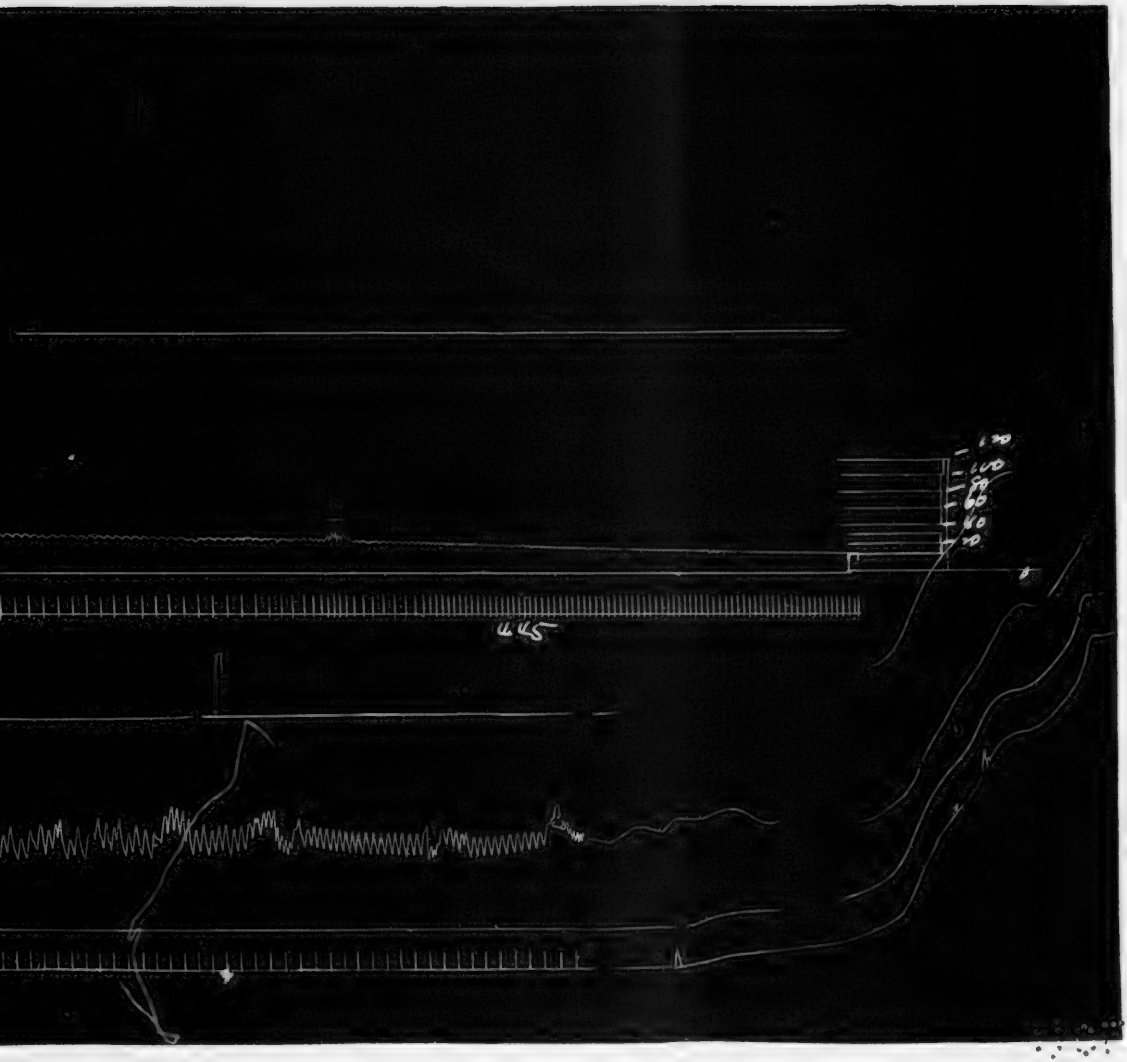


FIG. 2.

PLATE VII.



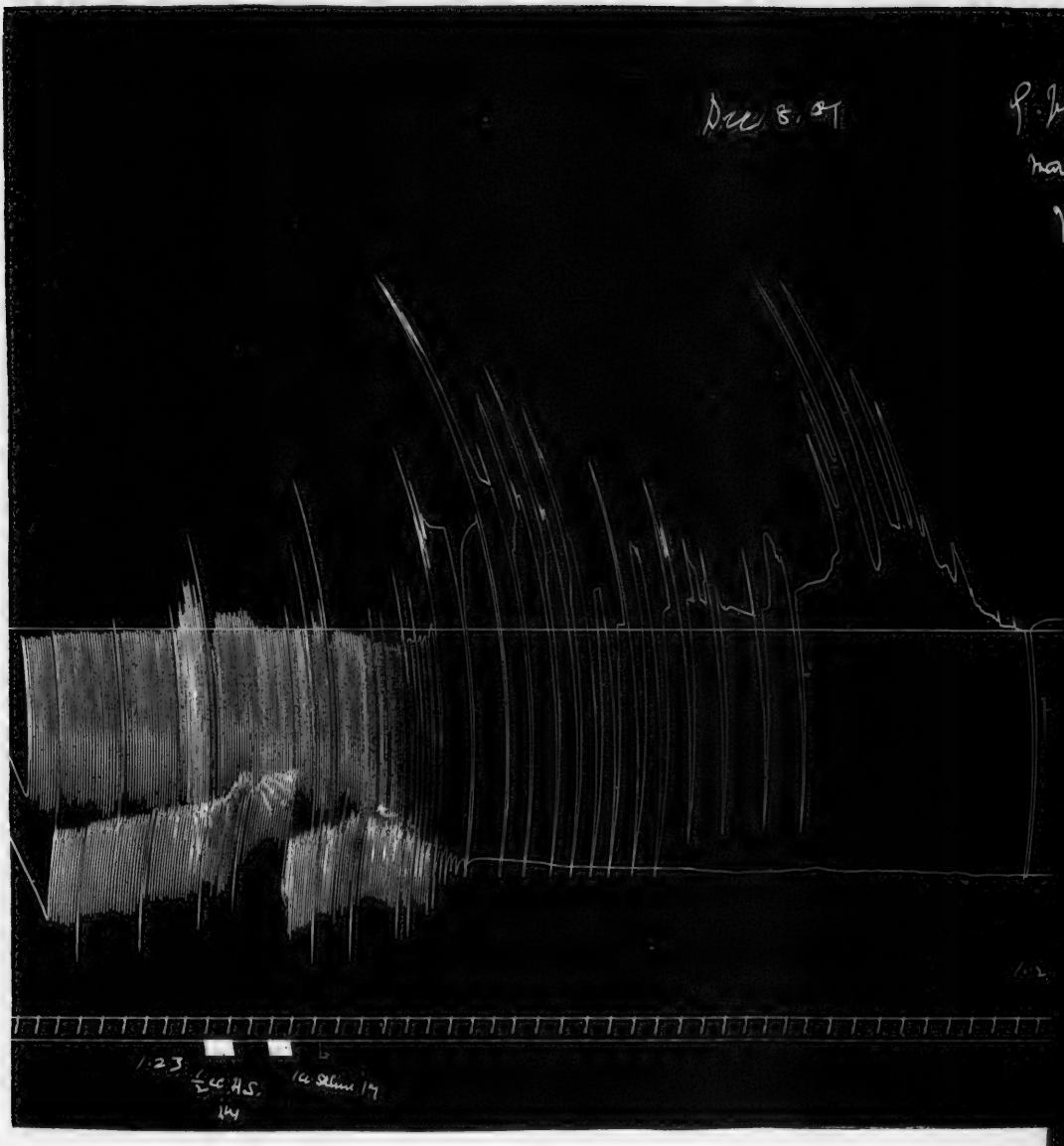
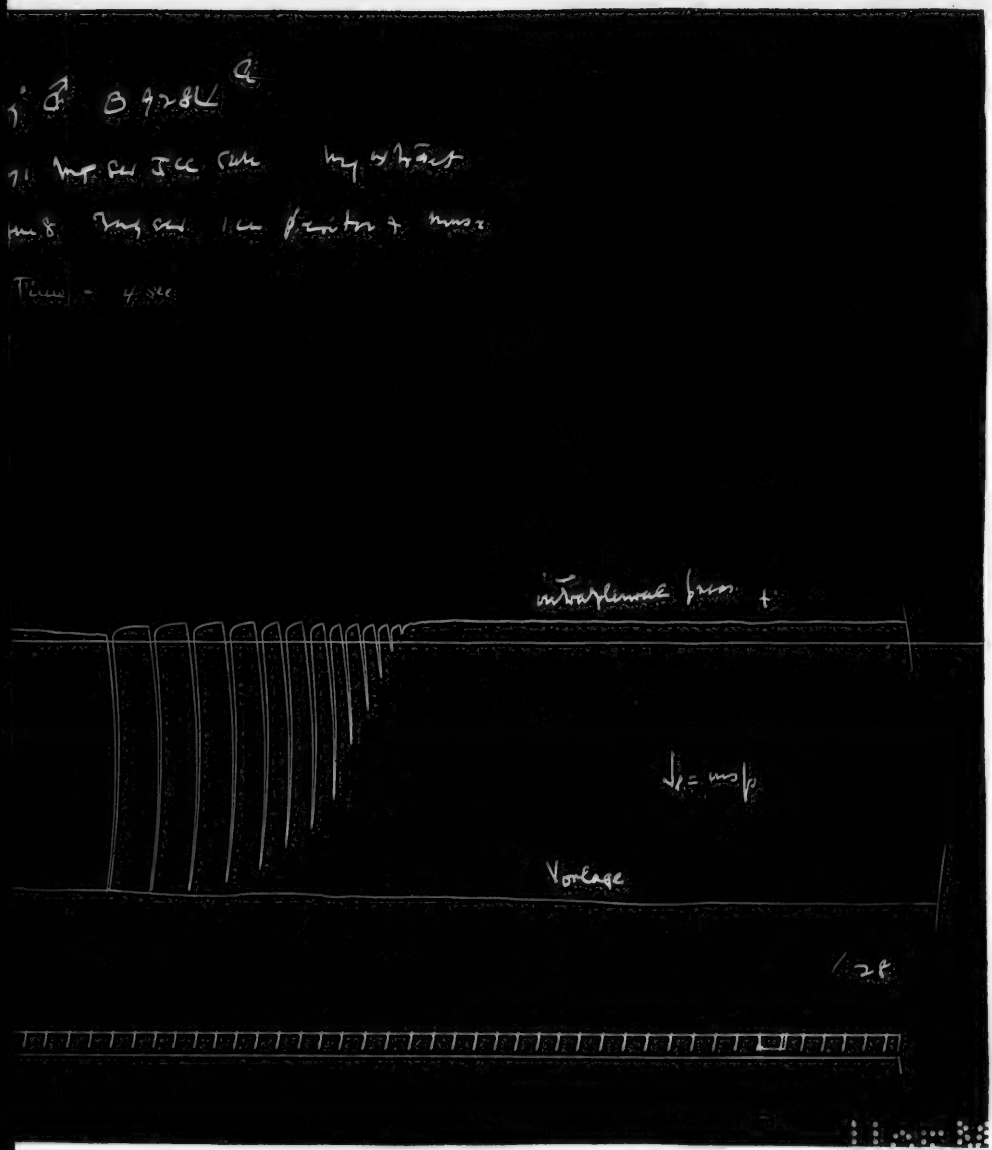
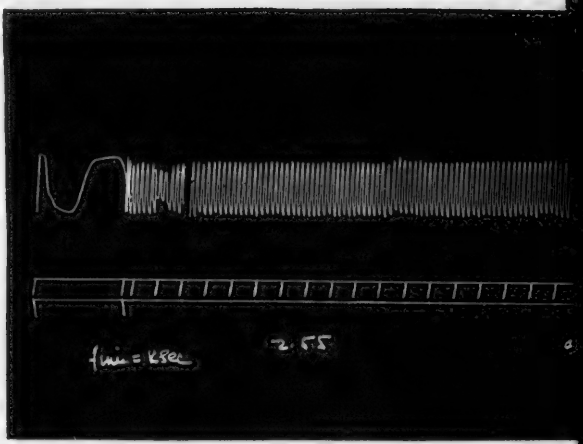


FIG. 3.

PLATE VIII.





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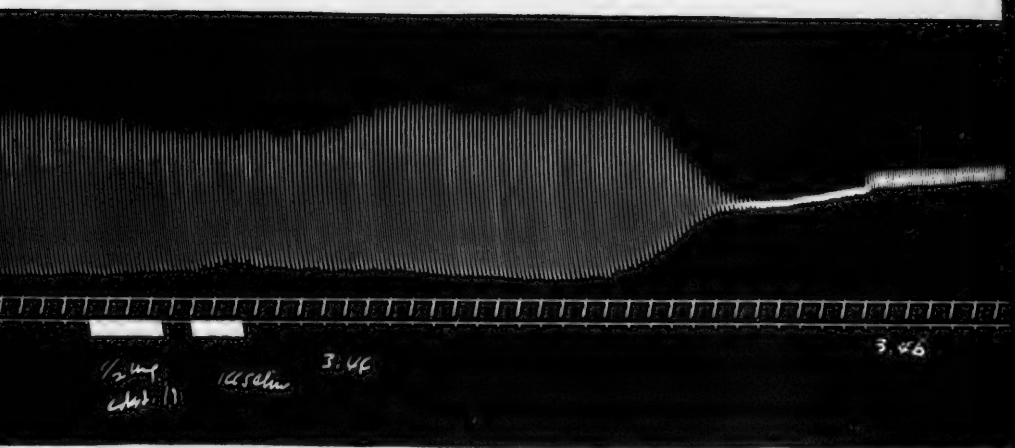


FIG. 5.

PLATE IX.

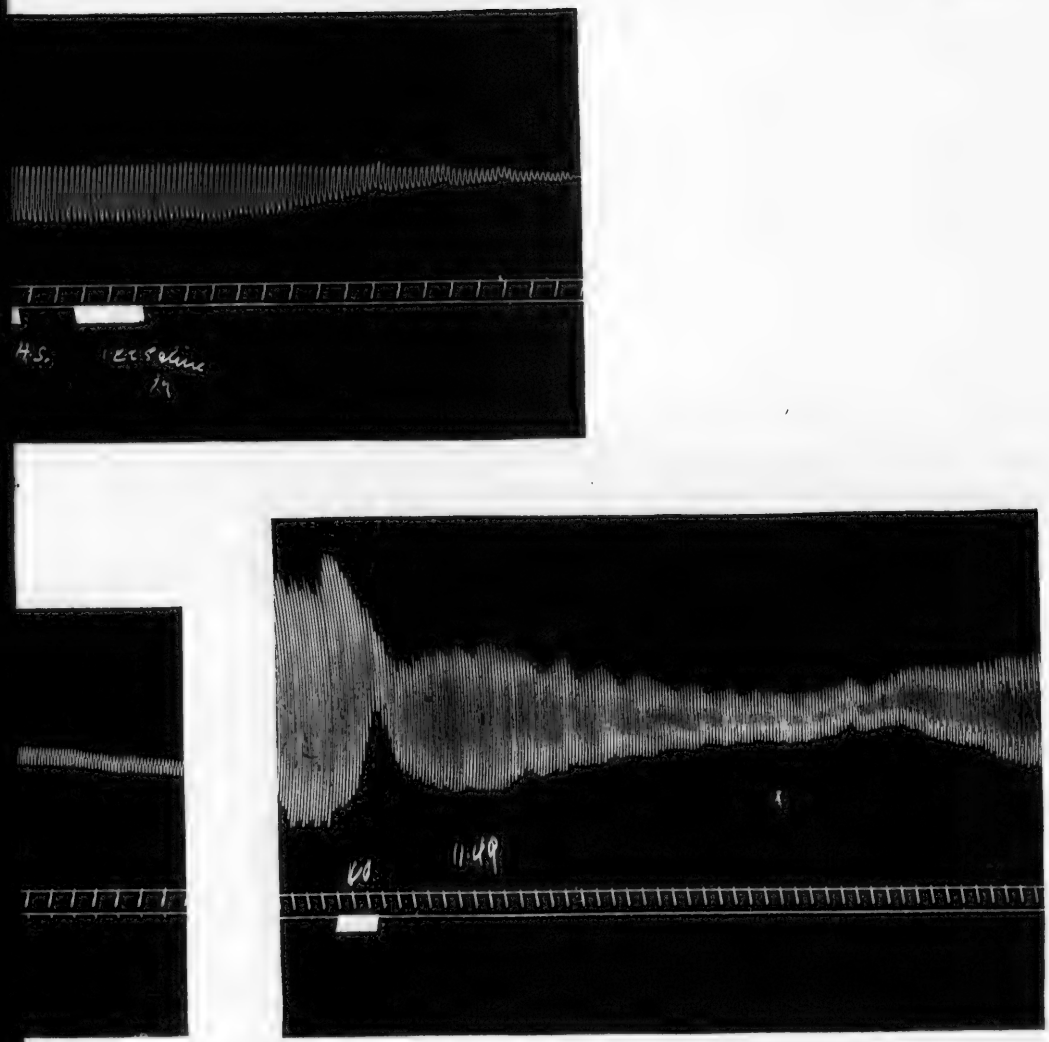


FIG. 6.

connected with the air bottle traces a straight line, while the intrapleural pressure curve shows that the animal makes active expirations and deep inspiratory *attempts*. The respiratory pause, preceded by a convulsion and followed by the terminal group of respiratory attempts is well brought out. At no point does the curve show an inspiratory tetanus of any duration. The straight line near the top of the curve represents atmospheric pressure. Above this line is positive pressure, below the line is negative pressure.

PLATE IX.

FIG. 4. This tracing was obtained from an animal whose cord, medulla, and basal portions of the brain had been destroyed by pithing. Artificial respiration was started before the pithing was completed and was recorded by means of a tambou fixed on the left side of the chest; upstroke equals inflation of the lung. At the places marked by broad white bands, 0.2 c.c. of horse serum and 1 c.c. of saline solution were injected into the external jugular vein. Shortly after this injection the lung volume oscillations increased slightly, then gradually decreased in amplitude until finally no oscillations were seen, although artificial respiration was still going on. The terminal oscillations also show increased slopes indicating slowness of air in entering and leaving the lungs. The lever finally comes to rest very near the inspiratory level of the curve, showing that the chest is considerably fuller than during expiration. Time line shows four second intervals.

FIG. 5. Tracing showing the volume changes of the lung in a pithed guinea-pig (cord, medulla and basal portions of the brain destroyed) obtained by means of a pleural cannula. Time is in four second intervals. At the places marked by broad white bands, 0.5 mm. of the edestin dissolved in N/20 sodium hydroxide, and 1 c.c. of saline solution were injected into the external jugular vein. (Artificial respiration was begun before pithing and steadily maintained; both vagi had also been stimulated by induction shocks to bring about a tonus of the bronchial muscles.) During and after these injections the lung volume changes caused by the artificial respiration became less; these gradually increased to more than before the toxic injections, then decreased again slightly, and finally decreased to such an extent that the artificial respiration caused no further changes in the lung volume. The small oscillations recorded now are due to volume changes of the heart; this curve of the heart beat shows abrupt changes in rate due to heart block.

This tracing shows graphically the peripheral attack of the toxic dose, and the rhythmic change in lung volume due to the action of the toxic dose upon the bronchial muscles.

FIG. 6. Tracing obtained from the pleural cavity of a curarized, sensitized guinea-pig. At the place marked by a broad white band both vagi were stimulated by an induced current (40 mm. coil distance; Petzoldt coil, 2 Daniell cells). Time is in four second intervals. The tracing shows well the rhythmic changes in lung volume which may be produced by stimulating the bronchial muscles through the vagi.

AN ATTENUATED SURRA OF MAURITIUS WITH IMMUNITY TESTS AFTER RECOVERY.*

By B. T. TERRY, M.D.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

Surra is so pathogenic for mice that in these animals, according to Laveran and Mesnil (see Nabarro[1]) "the disease, if untreated, always ends fatally." *To this statement no exception was seen while the surra of Mauritius*[2] *now to be described, was preserved in mice.* Under these conditions, injections similar to those in the first outline below, infected on the second or third day and killed on the sixth or seventh, while richer intraperitoneal inoculations infected on the first and usually killed on the fourth or fifth day.

Attenuated Mauritian Surra.—Subsequently, however, the virus was kept for six months exclusively in guinea pigs, and apparently as a result of the guinea pig passages, it became greatly attenuated[3] for mice. This is well shown in the outlines (page 179).

From the outlines we see that the mice[4] inoculated with the

*Received for publication January 24, 1910.

[1] Nabarro, D., Trypanosomes and Trypanosomiases. Translated from the French of Laveran, A., and Mesnil, F., Chicago, 1907, p. 265.

[2] This virus was placed at my disposal through the kindness of Dr. Felix Mesnil, of the Pasteur Institute, Paris.

[3] As far as I am aware, no one has described as great an attenuation of surra due to guinea pig passage, as that met with among my animals. Nevertheless it is known that the course of infection in mice may be somewhat altered when the virus is obtained from guinea pigs. According to Nabarro,[1] Laveran and Mesnil found that when once the parasites of Indian surra appeared, they never diminished except in a few cases in which the mice were inoculated with guinea pig blood.

[4] The two mice in the outline were controls on a mouse which had previously successfully resisted eleven inoculations with surra of Mauritius. When, however, this animal was tested with the attenuated strain, it was apparently slightly hypersensitive to infection, becoming positive on the fourth and dying on the ninth day after inoculation. The detailed history of this animal will soon be published.

attenuated strains were not visibly infected until the fifth day. From then until its death on the twentieth day, the first of these animals remained continuously infected. The second was for some weeks alternately positive and negative, then apparently recovered completely. In the blood of this animal in daily examinations, trypanosomes were found thirteen times in the first three weeks. Subsequently they were seen only on *the twenty-eighth, thirty-sixth and seventy-second day after inoculation.*

A Carrier of Trypanosomes.—The infection in the second mouse had been so persistent that, after the disappearance of the parasites, a careful watch for their return was kept for over six weeks. During this time trypanosomes were never detected and on the 121st day the mouse was still microscopically negative. As a more delicate test for the presence of the parasites, on this day ten to twelve drops of its blood were inoculated intraperitoneally into a normal mouse. The animal became infected and died, showing conclusively that *the mouse, in which the infection had been so persistent, had remained a carrier*[5] *of trypanosomes for seven weeks after the parasites had last been detected microscopically.*

A Spontaneous Cure.—It was now of considerable interest to determine whether the second mouse would continue to carry trypanosomes or would recover. To determine this point it was bled freely from the tail on the 130th and again on the 141st day, and all of the blood thus obtained was injected intraperitoneally into normal mice (into four at the first bleeding, into two at the second). In none of these animals, however, did infection take place, and three of the six were living 180 days later. In spite of the failure of the animal tests, microscopical examination of the blood of the second animal above was not discontinued until the 190th day, by which time it seemed certain that the mouse had recovered. It lived until the 217th day.

Two Other Spontaneous Cures.—In order to determine whether other animals would recover spontaneously, the following four mice received an injection of the strain of surra of Mauritius employed to infect the last two.

[5] For the production of trypanosome carriers in cattle by passing nagana (?) through rat and dog, the papers of R. Koch and C. Schilling should be consulted

After incubation periods of seven to sixteen days, all four animals became positive, two died infected and two apparently recovered. Animal inoculation, however, (see test on 50th day above) showed that *one of the last two mice remained a carrier of trypanosomes for over four weeks after the parasites were last seen in its blood.*

No Lasting Immunity after Recovery.—If mice could recover spontaneously, it was of interest to ascertain whether they acquired a lasting immunity. In order to test this point, on the 94th day the last two mice in the experiment above were reinoculated with the surra of Mauritius[6] from which they had apparently recovered. The result showed that when the test was made the animals pos-

Explanation of Outlines.—The outlines are read from left to right, each line representing a different mouse.

SM = an inoculation of surra of Mauritius. To the right above, in each instance, are indicated three points and in the following order: (1) The *quantity* of the virus introduced (.2 = two-tenths c.c., .3 = three-tenths c.c.); (2) the *mode of inoculation* (s = subcutaneous, i = intraperitóneal); (3) the *number of parasites* per field (Zeiss, lens "D," No. 4) in the injected suspension (in physiological salt solution).

The course of controls on the surra of Mauritius inoculations are shown by bold-faced figures to the right of the SM (see second outline, Mice 3 and 4, second inoculation with SM). The 2-7, 4-10 in this position mean that one control was positive on the second day after inoculation and dead on the seventh, and that the second control was positive on the fourth and dead on the tenth day.

° = no parasites in at least twenty fields. To save space some of the negative examinations are omitted.

+ = parasites (few or many) microscopically visible.

The *days* on which examinations were made are indicated by bold-faced figures placed above the zeros and plus signs.

L (large or small) = living.

D (large or small) = dead.

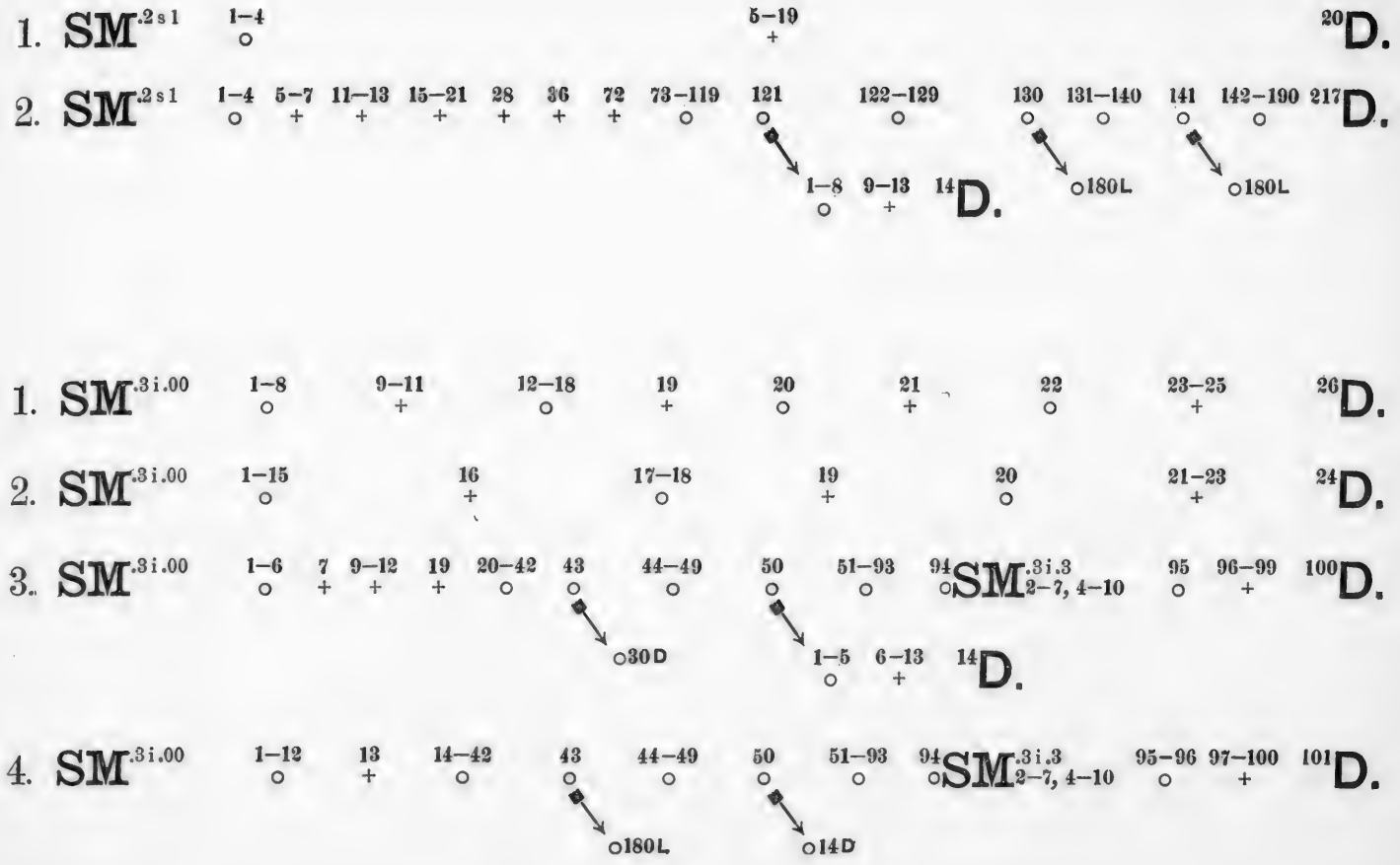
The *days* on which animals died or on which they were still living is shown by the figures placed to the left of the *L*'s and *D*'s.

° 180 *L* = negative and living on the 180th day.

° 30 *D* = remained negative, dead on the 30th day.

An *arrow* indicates a subinoculation and points from the animal supplying the blood to one receiving it. (The number of mice inoculated at one time is not shown. See text.)

[6] As inoculations of surra of Mauritius derived directly from guinea pigs had yielded uncertain results, before employing this virus in the immunity test it was passed through a single mouse to increase slightly its virulence.


1. SM.2s1
1–4
5–19
20D.
2. SM.2s1
1–4 5–7 11–13 15–21 28 36 72 73–119 121 122–129 130 131–140 141 142–190 217D.
1–8 9–13 14D.
180L
180L
1. SM.3i.00
1–8 9–11 12–18 19 20 21 22 23–25 26D.
2. SM.3i.00
1–15 16 17–18 19 20 21–23 24D.
3. SM.3i.00
1–6 7 9–12 19 20–42 43 44–49 50 51–93 94 SM.3i.3 2–7, 4–10 95 96–99 100D.
30D
1–5 6–13 14D.
4. SM.3i.00
1–12 13 14–42 43 44–49 50 51–93 94 SM.3i.3 2–7, 4–10 95–96 97–100 101D.
180L
14D

sessed no immunity, for both became infected promptly. They died six and seven days after inoculation; their controls, seven and ten.

Increased Virulence for Guinea Pigs.—As Laveran[7] had shown that the virulence of surra of Mauritius for guinea pigs might increase when the virus was preserved for a long time in these animals, it was of interest to see whether an increase of virulence for guinea pigs had not taken place in my virus. Since infected guinea pigs pursue very irregular courses, it was not possible to determine this point until the average length of life in groups of these animals had been calculated. As soon as this was done, however, it was perfectly obvious that, *while the parasites were being attenuated for mice, their virulence for guinea pigs had increased,* for the average life, in successive groups containing five or more of these animals, had grown progressively shorter. For example, on dividing the animals into groups of sixes, it was found *that the average life in the first group was 86 days; that in the second, 81; that in the third, 58.*

Virulence for Mice Restored.—From the result of the last experiment and from various published papers,[8] it seemed not improbable that the virulence of the surra of Mauritius parasites for mice could be restored by successive passages through these animals. In testing this possibility three experiments were carried out. In the first was employed the virus from which Mouse 2 (in the first outline) recovered. From this mouse the parasites for the second mouse passage were obtained by subinoculation on the 121st day. As had been expected, the virulence for mice increased in the successive passages, until, in the eighth mouse, the parasites developed as rapidly as before their attenuation. In the other two experiments the restoration of the virulence was even quicker as was well shown by the rapidly decreasing intervals between inoculation and death. In one of these series, for example, the first mouse died on the 16th day; the second, on the 11th; the third, on the 7th; and

[7] Laveran, A., Influence des passages par cobayes sur la virulence de quelques trypanosomes, *Bull. de la Soc. de path. exotique,* 1908, i, 198.

[8] For example, Laveran and Mesnil (see Nabarro[1]) have shown that the virulence of the Indian surra for the mouse may be greatly exalted by passage through rat or mouse.

the fourth, on the 4th day. In this experiment the normal level of virulence was reached after only four passages.

SUMMARY.

Apparently as a result of repeated passages through guinea pigs, the virulence of surra of Mauritius for mice became greatly attenuated. Three mice recovered spontaneously and *two remained carriers of tryanosomes for four to seven weeks after the parasites were last seen in their blood.* The mice that recovered acquired no lasting immunity. While the virulence of the parasites decreased for mice, it increased for guinea pigs. By cultivating the parasites in mice the virulence for these animals was restored after four to eight passages.

PLEOMORPHISM AND PLEOBIOSIS OF BACILLUS BIFIDUS COMMUNIS.*

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

Plates X–XIII.

Notwithstanding the extensive studies of Tissier,[1] Moro,[2] Rodella,[3] Cahn,[4] Passini,[5] Jacobson,[6] Herter and Kendall,[7] and a few others,[8] the relation between *B. bifidus communis* (Tissier, 1900) and certain other Gram-positive organisms of the feces, such as *B. acidophilus* (Moro, 1900), Kopfchenbacillus, *B. tuberculiformis intestinalis* (Jacobson, 1908) and *B. infantilis* (Kendall, 1909) remains still unsolved. At present it is difficult to say whether they are entirely different organisms, or whether they are different forms of one and the same organism. It is a recognized fact that the purity of a culture of *B. bifidus* cannot be guaranteed absolutely, as it often produces forms which differ in many respects from the original strain and which resemble, not infrequently, *B. acidophilus, B. tuberculiformis intestinalis,* or the Kopfchenbacillus. Moro abandoned his effort to identify the Kopfchenbacillus with *B. bifidus*

* Received for publication December 30, 1909.

[1] Tissier, *Compt. rend. Soc. de biol.*, 1899, li, 943; Recherches sur la flore intestinale des nourrissons, Paris, 1900; *Ann. de l'Inst. Pasteur,* 1905, xix, 109.

[2] Moro, *Jahrb. f. Kinderh.*, 1900, lii, 38; *Wiener klin. Woch.*, 1900, xiii, 114; *Jahrb. f. Kinderh.*, 1905, lxi, 687.

[3] Rodella, *Cent. f. Bakt, I Abt., Orig.*, 1901, xxix, 717; *Zeit. f. Hyg.*, 1902, xxxix, 201; *Cent. f. Bakt, I Abt., Orig.*, 1903, xxxiv, 14.

[4] Cahn, *Cent. f. Bakt, I Abt., Orig.*, 1901, xxx, 721.

[5] Passini, *Jahrb. f. Kinderh.*, 1903, lvii, 87.

[6] Jacobson, *Ann. de l'Inst. Pasteur,* 1908, xxi, 303.

[7] Herter and Kendall, *Jour. of Biol. Chem.*, 1908, v, 289; Kendall, *Jour. of Biol. Chem.*, 1909, v, 419.

[8] Finkelstein, *Deutsche med. Woch.*, 1900, xxvi, 263; Cipollina, *Cent. f. Bakt, I Abt., Orig.*, 1902, xxxii, 576; Weiss, *Cent. f. Bakt, I Abt., Orig.*, 1904, xxxvi, 13.

because of his inability to produce a bifurcating strain from the former, while Jacobson met a similar failure in his attempt to reverse *B. tuberculiformis intestinalis* into *B. bifidus.* In spite of numerous attempts, as yet no one has succeeded in tracing the source of *B. bifidus* outside of the intestinal tract.

In the course of my study of anaerobic bacterial flora of the intestinal tract of healthy and diseased children, my attention was directed to *B. bifidus.* The results I obtained from my present study on *B. bifidus* convinced me that *B. bifidus communis* of Tissier is an anaerobic phase of life of an aerobic sporogenous organism belonging to the subtiloid group and closely resembling, especially morphologically and biologically, *B mesentericus fuscus.* By certain cultural methods, I was able to induce sporulation and adaptation of the aerobic life of *B. bifidus* and then lead the aerobized *B. bifidus* back to the anaerobic bifurcating phase. In the aerobic phase of *B. bifidus,* no bifiurcation has been observed, and it seems almost incredible that it should be related to anaerobic *B. bifidus* at all. With adaptation of aerobiosis and anaerobiosis the entire sets of morphological and biological characteristics undergo profound alterations. There are intermediate phases between these two extremes, which give the bacillus characteristics of semi-anaerobiosis and extreme morphological variabilities.

The following experiments were made. Eight pure colonies of *B. bifidus,* two of which consisted of a somewhat more delicate type of bacillus, and one pure colony of a bacillus resembling a type of *B. acidophilus* were isolated from the fresh stool of a healthy, breast-fed girl, aged two months, by means of glucose-agar plating in an anaerobic apparatus constructed on the same principle as that of Schattenfroh and Grassberger. The purity of these colonies was ascertained first by microscopical examination and then by culture. For the latter purpose, I found it advisable to use high layer agar containing 1.5 per cent. lactose or glucose, because with this bifurcation was more general and uniform than with any other liquid media for which an anaerobic apparatus is required. Even with the use of fresh tissue in bouillon or glucose bouillon, *B. bifidus* was seen to bifurcate less constantly than in high layer sugar agar,

and to produce a pleomorphic condition[9] which makes it difficult to determine the purity of an organism. The bacillus which appeared like *B. acidophilus* in colony turned out in high layer glucose agar to be a regular bifurcating strain. On the other hand, a typical bifidus strain often grew out of colonies with some bifurcating and non-branching acidophilus types, giving the appearance of mixed colonies.

SPORULATION OF B. BIFIDUS COMMUNIS.

Inoculations with these nine strains were made into solid and liquid media. The organisms were inoculated in the melted state, or by stab, into the high layer agar containing lactose or glucose (1.5 per cent.) and were cultivated at 37° C.; the inoculations into liquid media (litmus milk, beer wort bouillon, glucose bouillon, and especially Hiss's serum water, containing different sugars) were cultivated usually for seven days before examination in an anaerobic apparatus at 37° C.

The agar cultures were examined from time to time and in most cases I found bifurcation within twenty-four hours. As the bacilli grew older, the number of bifurcations and the length of the branches increased, and at the same time lost their ability to stain with Gram's method (Figs. 1, 2, 3, 4, 5).

The lactose- and glucose-serum-water were suitable media for *B. bifidus* and the sugars were fermented, without gas production, up to coagulation of the serum (acid production). Microscopical examination showed the presence of pleomorphic, non-branching forms (diplobacilli with tapering ends, spindles, short rods, beaded forms, coccobacilli, and a few bifuracted specimens). Vesicular forms were also seen occasionally. Besides these, I observed occasional free or attached oval spores (Figs. 6, 7, 8, 9).

By cultivating in succession pure strains of *B. bifidus* in sugar containing agar by my modification of the Marino plate,[10] I was

[9] There are in such cultures usually non-branching forms, including delicate bacilli with tapering ends, club-shaped, beaded, or tad-pole forms. Bifurcation is rare. In beer wort bouillon, delicate bifurcation may sometimes be produced.

[10] Marino, *Ann. de l'Inst. Pasteur*, 1908, xxi, 1005. I made two narrow breaks on opposite sides of a small Petri dish. I then placed it, with the opening downwards, in a larger Petri dish so that the liquid agar could flow from the large dish into the smaller one.

able to obtain, at intervals of two or three weeks, colonies in which a few straight Gram-positive bacilli were seen side by side with Gram-negative, bifurcated forms apparently of a degenerating stage. A few spores were often seen in such cultures (Figs. 10, 11, 12, 13).

The spore-forming organisms of these old cultures of *B. bifidus* were isolated by the usual methods. The cultures were heated to 100° C. for five minutes and plated out with modified Marino plates or diluted in high layer agar containing lactose and glucose.

From each strain I obtained two distinct strains of bacilli, which differed in their sensitiveness to oxygen, and also slightly in their morphological and cultural characteristics. One of the two was strictly aerobic, while the other was a facultative anaerobe. The first produced felted, dry colonies and contained a spore-forming, Gram-positive, motile bacillus, while the second formed rather moist, greyish-white colonies and the bacilli were somewhat more slender than those of the first. I found, however, that the second variety becomes gradually strictly aerobic and like the first variety after a few successive aerobic cultivations (Figs. 14, 15, 16, 17).

In the following protocols, I have given detailed descriptions of these two varieties of bacilli which have sprung from apparently pure cultures of *B. bifidus* under certain circumstances. The morphology of these two strains will be given in Table I which follows the experiments on reversion.

CULTURAL CHARACTERISTICS.

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
Agar plate plain agar.	24 hours at 37° C.—The colonies are very small, irregularly round or oval, and rather opaque. Diameter less than 1 mm. Under low power magnification: edge fairly well defined with curled filaments projecting from the entire circumference, some as long as the diameter of the colony. These curled filaments are irregularly interwoven, but sparse. Struc-	24 hours at 37° C.—The colonies are round or oval, irregularly contoured, elevated, finely granular, faintly grayish-brown, dimly edged, opaque, some containing thick centers. Deep colonies roundish or lenticular. By reflected light, grayish-white and shiny. Diameter of single colonies about 1 mm. 4 days at 37° C.—*A felted edge* is formed around the colonies

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
	ture: yellowish-brown, granular appearance due to the interwoven curved filaments without distinctive nucleus. By reflected light the colony looks grayish-white, dry, raised, and of irregular surface. 72 hours at 37° C.—The colonies are about 6 mm. in diameter, and show typical felted appearance.	without changing smoothness of the latter.
Glucose agar.	24 hours at 37° C.—The size of colonies is somewhat larger than that of plain agar colonies. Irregularly round or oval. Opaque, yellowish-brown and darker than in plain. Edge irregularly serrated, fairly well defined, and projecting from it many uneven, almost transparent, finely granular, wart-like outgrowths. Outer contour is surrounded by a lamellated marginal zone. This peripheral zone is caused by mucilaginous secretion of the colonies. By reflected light the colonies look grayish, pulvinated, with dried edge and moist center, which give a pearl-like appearance. Corrugated structure. 72 hours at 37° C.—Many colonies sent out typical felted filaments.	24 hours at 37° C.—Much the same as colonies on plain agar, but there is mucin production and often the colonies have a felted edge of long filaments comparable with the aerobic phase colonies. 4 days at 37° C.—Growth more vigorous but no other essential change.
Agar stab plain agar.	24 hours at 37° C.—Filamentous growth which is better nearer the surface. 48 hours.—Slight outgrowth along the stab near the surface. 7 days.—Growth covered the surface with thick membrane. 30 days.—Browned the media.	24 hours at 37° C.—Filiform growth along entire stab. There is a slight surface growth out of the puncture. 48 hours.—Slight surface growth. 7 days.—No further change. 30 days.—Browned the media slightly.

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain)
Glucose agar.	24 hours at 37° C.—Thin, uniform, smooth, filiform growth. Surface outgrowth is abundant. 48 hours.—Little outgrowth near the surface. Thick, mucoid, grayish membrane covered the surface. 30 days.—Browned the media.	24 hours at 37° C.—Similar to the above. 30 days.—Slightly browned the media.
Melted agar—inoculation.	24 hours at 37° C.—Growth obtained only on the surface, none in the deeper layer.	24 hours at 37° C.—Small punctiform colonies throughout the entire agar column. No gas formation. Surface is covered with thin layer of colony.
Plain agar.	After a few days the colony became thick, grayish, wrinkled, tough. 30 days.—Some root-like colonies grown into the upper layer of the agar. Browned the agar.	30 days.—Apparently no change, except browning the media.
Glucose agar.	Similar to the above, but more mucoid in character. 30 days.—Browned the agar.	24 hours at 37° C.—Somewhat better growth in the layer nearer the air. 30 days.—Apparently no change, except browning the media.
Agar stroke plain agar.	24 hours at 37° C.—Abundant, broad, spreading, rather flat, slightly more elevated in the middle, and firmly adherent, rugose, opaque, felt-edged colony. Grayish-white, in general dry, but beset with minute glistening droplets. Not larger than 1 mm. By transmitted light, the margin and center distinctly defined, the margin being dense and opaque—about 4 mm.; the center is less opaque and is finely wrinkled. Condensed water is slightly turbid and has thin, imperfect, grayish-white film. Medium unchanged.	24 hours at 37° C.—Moist, grayish-white, smooth-edged, but quite zigzag near the condensed water, where it spreads. Condensed water is turbid, *without* a film. Medium unchanged. 4 days at 37° C.—The edge of the colony assumed a zigzag appearance.

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
	72 hours at 37° C.—Growth advanced.	
Glucose agar.	24 hours at 37° C.—Spread over all surface. Raised by the accumulation of mucoid droplets which are almost transparent. Entire surface is covered with mucus drops of varying sizes. Medium unchanged. Colonies difficult to scrape from agar. 72 hours at 37° C.—Began to present more felted appearance and to show a tendency to get dry.	24 hours at 37° C.—Spreading, grayish white, undefined edge with numerous outgrowths projected from it. There is tendency to confluence. The colony is rather flat and very slightly elevated. The condensed water is cloudy. Medium somewhat turbid. 4 days at 37° C.—Slowly growing, but no felted appearance developed. The colonies do not adhere to the agar firmly, but can easily be removed with loop. The consistence is sticky.
Litmus milk.	24 hours at 37° C.—Color turned red, and a heavy yellowish-white deposit is formed on the bottom. 48 hours.—More yellowish. 72 hours.—Coagulated. 30 days.—Much of the coagulated casein dissolved. Reaction is slightly acid.	24 hours at 37° C.—Reddened on the top, and deeper layer decolorized. 7 days:—Coagulated. 30 days.—Coagulation seems to have dissolved somewhat. The reaction remains acid.
Loeffler's serum.	24 hours at 37° C.—Grayish-white, rugose, spreading colony. The substance beneath the colony appears to be depressed. There are mucoid droplets on the colony. 48 hours.—Liquefaction already set in and advancing. 4 days.—Not completely liquefied. 30 days.—Lower portion of the medium almost entirely dissolved. Brownish.	24 hours at 37° C.—Whitish-gray, shiny, elevated, wavy edged, no depression of the medium. 48 hours.—No definite liquefaction. 4 days.—No further change. 30 days.—Somewhat sunken along the colony, indicating a feeble power of proteolysis.
Gelatin.	24 hours at 37° C.—Minute flocculi in clear medium. On the surface are floating grayish-	Much the same as the aerobic phase, but the liquefaction is decidedly slower.

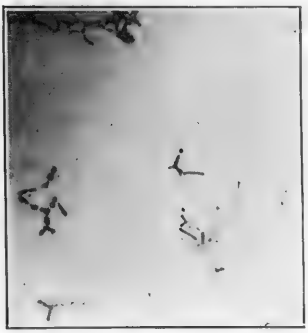


FIG. 13.

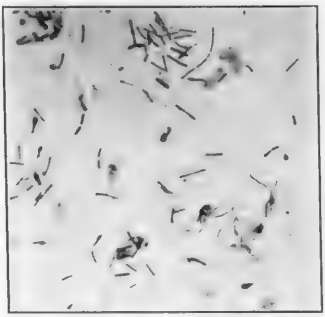
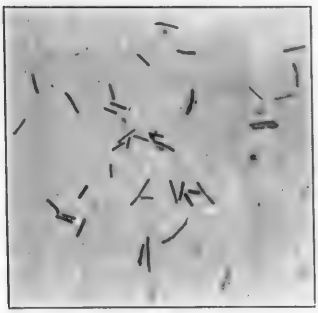


FIG. 14.



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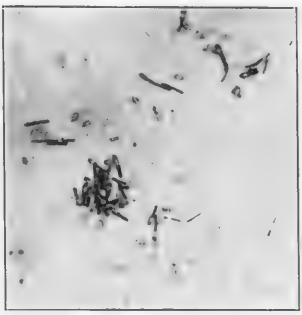

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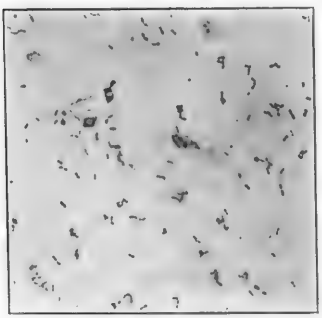

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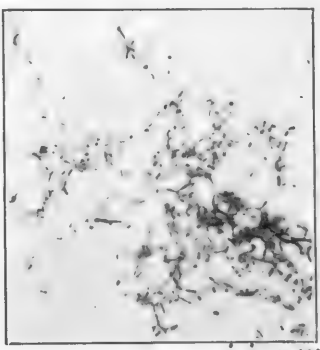

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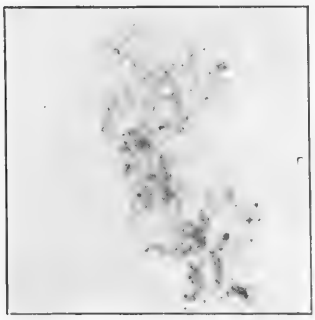
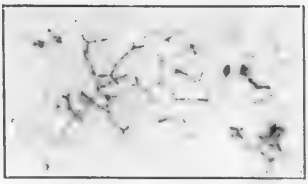


FIG. 19.



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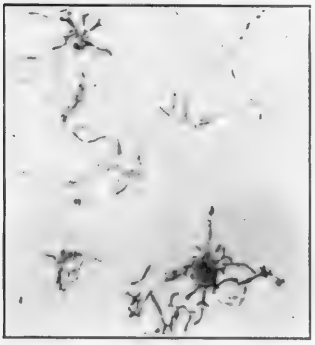

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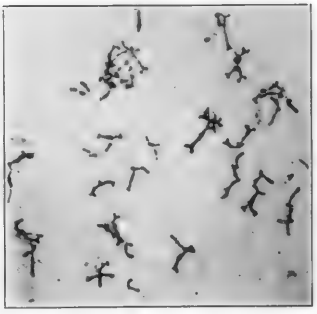

FIG. 22.

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	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
	white, dull, irregularly contoured pieces of colonies. 72 hours at 37° C.—Scum is formed. 7 days.—Liquefaction apparent. At 20° C.—Very limited amount of liquefaction of gelatin along the stab puncture in 2 weeks. 30 days.—Moderate liquefaction. Slight surface growth. In the stab there are many lateral projections of fine growth near the surface. Liquefaction is about 1 mm. deep and 6 mm. in diameter.	At 20° C.—Liquefaction is slight after 30 days. Growth is also poorer.
Plain bouillon.	24 hours at 37° C.—Slight turbidity with strong surface membrane, which sinks when torn, with the exception of narrow annulæ around the surface on the tube-wall. On vigorous shaking it breaks up into a fine flocculence. 48 hours.—No new scum. 72 hours.—A new, firm, wrinkled, whitish-gray membrane over the surface.	24 hours at 37° C.—Slightly turbid, with slight whitish sediment. No surface membrane. 72 hours.—No further change, except more turbidity and deposit.
Glucose bouillon.	24 hours at 37° C.—More turbid and finely granular sediment than in plain bouillon. On shaking, the sediment diffuses. No surface membrane except the annula. 48 hours.—Distinctly acid. 72 hours.—Firm surface membrane formed.	Similar to the above. Some acid production. No scum.
Potato.	24 hours at 37° C.—Grayish-white (faintly brownish at the margin of the colony), wrinkled, thick, scaly, dull, spreading. The colony is adherent to potato, but is quite	24 hours at 37° C.—Growth on potato *invisible*, with the fluid very turbid. 4 days.—No visible surface growth. 30 days.—No further change.

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
	moist and brittle (suggestive of colonies *B. tuberculosis* on veal bouillon-glycerin agar slant). 48 hours.—Color intensified and colony thicker and spreading. 72 hours.—Distinctly brownish-gray, somewhat reddish in places. Thickened and heavily wrinkled. Scum on the fluid. 4 days.—Brownish and dirty hue. 30 days.—Dark, brownish, gray hue.	

The descriptions given of the aerobic strain of the two organisms agree closely with those of *B. mesentericus fuscus.*

REVERSION OF AEROBIC PHASE OF B. BIFIDUS COMMUNIS INTO ANAEROBIC PHASE.

In the foregoing pages I described "the springing out" of two varieties of spore-forming, non-branching aerobes from certain cultures of *B. bifidus.* This finding might, of course, be considered as a gross contamination with the brown potato bacillus, had I not been able to produce all typical vegetative forms characteristic in every detail of *B. bifidus communis* from the spore material heated to 100° C. for five minutes of these two strains. However, by gradual training of the organisms to anaerobic life, I was able to accomplish complete reversion of the aerobic phase of this bacillus into the anaerobic. I cultivated the bacilli first semi-aerobically, abruptly diminishing the quantity of oxygen. After three or four successive cultivations, I obtained anaerobiosis in which condition the organism had grown well. Glucose bouillon and Hiss's serum water containing different carbohydrates, especially lactose, dextrin, inulin, saccharose and amygdalin, were found to be very suitable for reversing the biological phase. Anaerobic condition was produced usually by hydrogen gas, although nitrogen, carbon dioxide and methan were equally suitable for the production of typical bifurcating forms. The degree of reversibility of the strictly

aerobic variety is found to be inferior to that of the semi-aerobic strain, and more generations are required before complete reversion is attained.

During the reversing processes, I observed that the first step of reversion of the aerobic spore-material towards anaerobiosis is characterized by the appearance of streptococcal or young staphylococcal forms with, occasionally, *minute* forms of bifurcating types. In the next stage the numbers of coccobacillary forms gradually diminish and more typical bifurcating bacilli of *regular* size and shape, with many short primitive types of bifidus, appear. In this stage, the developmental steps from coccal forms to regular bifidus are clearly seen (Figs. 18, 19). Stellated arrangements of young bifurcating rods are often seen.

A further step towards anaerobization shows the tendency to form more bifurcated bacilli of regular size, and in the stage following this phase, we obtained *B. bifidus communis* in the sense used by Tissier (Figs. 20, 21, 22).

The fermentative faculty of *B. bifidus* and its aerobic varieties were tested upon different kinds of sugars and glucosides, but the results were extremely inconstant. In one series of experiments they split almost every sugar employed with acid production and, on other occasions, they did not attack any of them. I found, however, that in the strictly aerobic phase of the bacillus it is rare to get coagulation of serum or milk, while in semi-aerobic and strictly anaerobic phases, acid-production seems to cause coagulation of these proteids in several days. This difference may be due to simultaneous proteolysis in the aerobic phase. Table I describes summarily the morphology of the four different phases of *B. bifidus*.

CONCLUSIONS.

From the foregoing experiments, the conclusion may be drawn that *B. bifidus communis* of Tissier has an aerobic phase, in which it resembles *B. mesentericus fuscus*. Numerous intermediate phases can occur between these two extremes; and their morphological and biological variabilities demand the utmost attention in order to interpret more intelligently the various phases of a given organism, constantly found in the stools of sucklings, and to avoid the artificial creation of two or more organisms from a single microbic type.

TABLE I.

MORPHOLOGY.

	Size.	Chains.	Threads.	Pairs.	Motility.	Sporulation.	Gram's Stain.
Aerobic phase (obligatory). The 1st strain belongs to this phase.	Length, 1.5 μ to 5 μ. Width, 0.3 μ to 0.6 μ.	More frequent in bouillon, some covering entire field. In old cultures more than in the new.	Rare, but may be seen in old liquid culture, often quite long, equalling half the field. 20 to 50 bacilli may be seen. Almost none in young agar culture.	2, seldom 3 or 4, parallel, occur quite frequently.	Very active serpentine, rotating, waltzing. Flagella at both ends.	Occurs within 24 hours in almost every case. Polar or middle spores, oval, spore membrane noticed, especially at the antipole. Easy detachment from the bacilli. Length, 0.8μ to 1.5 μ. Width, 0.5 μ to 0.8 μ.	Young cultures usually uniformly positive. After 2 to 4 days the staining becomes less intense and decolorized more easily by alcoholic treatment.
Facultative anaerobic phase (tendency toward aerobic). The 2d strain belongs to this phase.	Length, 2 μ to 5 μ. Width, 0.3 μ to 0.6 μ.	There are very few branching forms. There are forms of straight, round ended, or somewhat curved diphtheria bacillus-like forms Some give the appearance of tubercle bacilli.		Pairs are often present.	Some are quite actively and many are slowly motile. Many dancing, waggling and rotating. Flagella at both ends.	Early sporulation occurs in the straight forms, but only in very old cultures does it occur in other types. Almost always polar. Shape and size same as above. (Fig. 16.)	There are various specimens with different degrees of staining by Gram's method. The straight forms stain more intensely.
Facultative anaerobic phase (tendency toward anaerobic).	Length, 1.5 μ to 5 μ. Width, 0.3 μ to 0.6 μ. In reversing experiments from the spores coccobacillary forms or punctiforms often appear. They are much smaller in size and more pleomorphic than the phase arising from the anaerobic bifidus. (Figs. 17 and 18.)	Geniculated non-branching forms predominate. The ends may be pointed or thickened to suggest the bifurcation. There may be a few branching forms resembling tubercle bacilli or dotted bacilli like diphtheria bacilli.		Pairs of non-branching forms are frequent.	Some slowly motile. Others immobile.	Fewer or no spores are produced by the young cultures, but almost always some are formed in the old semi-aerobic cultures. Always polar. Shape and size same as above.	Young cultures quite uniform; sometimes complete decolorization in a short time.

	Size.	Chains.	Threads.	Pairs.	Motility.	Sporulation.	Gram's Stain.
Anaerobic phase (obligatory) or *B. bifidus communis* of Tissier.	Length, 1.5 μ to 5 μ. Width, 0.3 μ to 0.6 μ. From a continuous bunch of bifurcated bacilli, in old lactose agar, one projection measured about 10 μ.	Geniculation of branching forms twice or many times is common in old solid cultures. There are examples of sternata arrangement of bifurcating forms. This is more frequent in the reversing of spores into anaerobic phase. Straight forms may appear in old sugar agar cultures.		In young, non-branching cultures, 2 to 4 or even more, parallel to each other, occur. Bifurcating forms do not pair so often.	Motility doubtful, perhaps absent.	While young, there is no sporulation, but spores appear in straight bacilli in old cultures under semi-anaerobic preservation. Always polar, often free. Shape and size same as above.	Young cultures quite uniform; the bifurcating portion of branches decolorize much more easily than the other parts. Gram's stain becomes irregular when cultures get old, and may decolorize completely.

The frequent occurrence of *B. mesentericus* in the stools of sucklings has been described by Tissier, Moro, and many others. That *B. mesentericus* is one of the most wide spread saprophytes and is constantly found on the surface of our skin, is a well established fact. Scheurlen[11] was once led to consider this bacillus as the cause of carcinoma, because he isolated it from the cancers of mamma (interior of the tumors). But Rosenthal[12] found the same organism in the breasts of healthy persons.

I, therefore, consider that the one source of *B. bifidus communis* in the stools of breast-fed infants is the breast of the lactating mother.

EXPLANATION OF PLATES.

Plate X.

Fig. 1. Non-branching forms of *B. bifidus communis.* From a young culture in high layer glucose agar.

Fig. 2. The beginning of bifurcation of *B. bifidus.* (18-hour-old glucose agar stab culture.)

Fig. 3. Pleomorphic state of young *B. bifidus* culture, showing the tridents, *Y*-forms, wedges, geniculations, non-branching individuals, etc. From a young, lactose agar high layer culture (48 hours old).

Fig. 4. Multiple bifurcations of *B. bifidus.* From an old, high lactose agar culture.

Fig. 5. Multiple bifurcations of *B. bifidus* showing abnormally large individual with enormously long projections. From an old, high glucose agar culture.

Fig. 6. Sporulation of *B. bifidus.* A spore-bearing short bacillus with two individuals is seen on the left. Straight, curved, or geniculated forms are also seen, but very few bifurcated forms. This shows the stage of springing out of aerobic individuals from an old culture of *B. bifidus.* From an old inulin-serum water culture.

Plate XI.

Fig. 7. Non-branching phase of *B. bifidus.* Diplobacilli forms, candle-flame forms, banded and striated forms and many other forms are seen. From 7-day old lactose serum water culture.

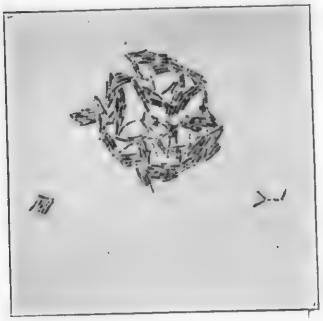
Fig. 8. Pleomorphic feature of *B. bifidus.* From a young saccharose serum water culture.

Fig. 9. Sporulation of *B. bifidus.* Besides Gram-negative bifurcated forms, there are several Gram-positive straight bacilli which represent the semi-aerobic phase of *B. bifidus.* From an old culture in modified Marino plate.

Figs. 10 and 11. Sporulation of *B. bifidus.* From a 14-day-old lactose agar plate after modified Marino method.

[11] Scheurlen, *Berliner klin. Woch.*, 1887, xxiv, 935.

[12] Rosenthal, *Zeit. f. Hyg.*, 1889, v, 161.



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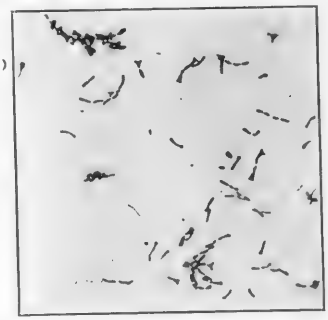

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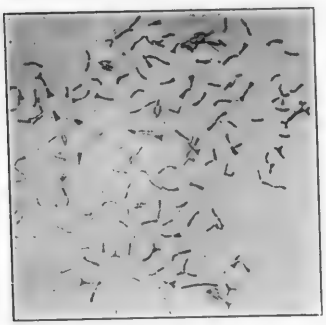

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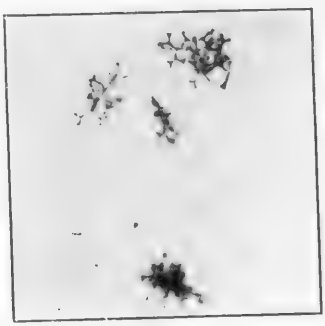

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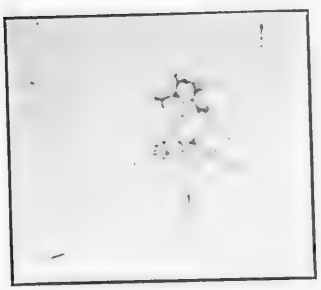

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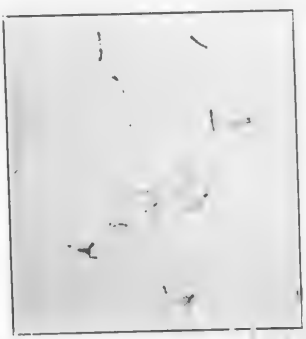

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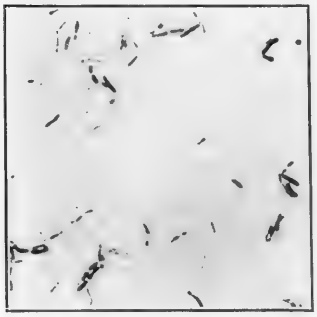

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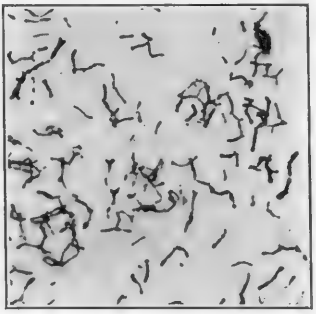

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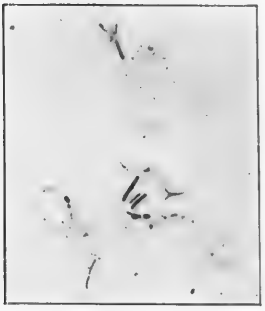

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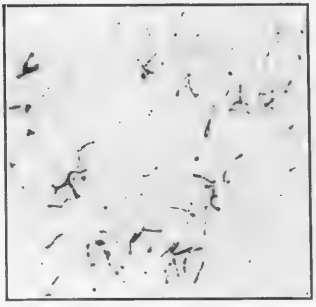

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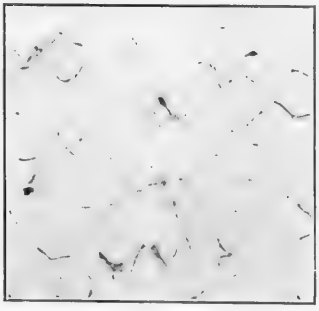

FIG. 11.

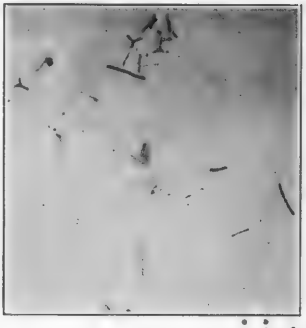

FIG. 12.

FIG. 12. Transition of bifurcating forms to straight type. From old modified Marino plates.

PLATE XII.

FIG. 13. Transition of bifurcating forms to straight type. From old modified Marino plates.

FIG. 14. Immediate semi-aerobic generation of *B. bifidus* just sprung from the anaerobic phase and showing transitory forms from non-branching anaerobic to straight sporulating phase. From a 24-hour colony in the deep layer of lactose agar near the anaerobic sphere.

FIG. 15. Semi-aerobic phase of *B. bifidus*. From a 2-day-old surface colony on glucose agar plate.

FIG. 16. Sporulation of semi-aerobic phase of *B. bifidus*. From a 3-day-old agar plate colony grown aerobically.

FIG. 17. Reversion of aerobiosis into anaerobiosis. Coccic stage with a few minute, bifurcated forms. From a semi-anaerobic cultivation of aerobic phase in glucose bouillon for 6 days at 37° C.

FIG. 18. Reversion. A step nearer anaerobiosis than the foregoing coccic stage. Extremely pleomorphic.

PLATE XIII.

FIG. 19. Reversion almost completed. From the third generation in reversing cultivation. Six days in glucose bouillon.

FIG. 20. Reversion completed. From dextrin serum water culture. Anaerobically cultivated.

FIG. 21. Reversion completed. Still numerous irregular types.

FIG. 22. Reversion completed. Very regular *B. bifidus* forms. From a lactose bouillon culture. Third anaerobic generation of the aerobic strain in glucose bouillon for 6 days at 37° C.

EXPERIMENTAL ACUTE NEPHRITIS: THE VASCULAR REACTIONS AND THE ELIMINATION OF NITROGEN.[1]

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PLATES XIV–XVII.

This investigation is one of a series suggested by the studies which Schlayer and his associates[2] have made of various forms of experimental nephritis in the rabbit, and has for its object the determination of the types of such nephritides in the dog, with special reference to the involvement of tubules and vessels (glomeruli) respectively. Physiological, chemical and anatomical methods have been employed in the attempt to demonstrate the distinctive types of lesion.

While the investigation is, in a way, a repetition of Schlayer's work, it has other features of interest. The dog has been selected instead of the rabbit, partly because this animal can be more readily catheterized thus permitting a more definite study of conditions

[1] Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication January 6, 1910.

[2] Schlayer and Hedinger, Experimentelle Studien über toxische Nephritis, *Deutsch. Arch. f. klin. Med.*, 1907, xc, 1; Schlayer, Hedinger and Takayasu, Über nephritisches Ödem, *ibid.*, 1907, xci, 59; Takayasu, R., Ueber die Beziehungen zwischen anatomischen Glomerulus-veränderungen und Nierenfunktion bei experimentellen Nephritiden, *Deutsch. Arch. f. klin. Med.*, 1907, xcii, 127; Schlayer and Takayasu, Untersuchungen über die Funktion kranker Nieren. *Münchener med. Woch.*, 1909, lvi, 2201.

of anuria and polyuria, and partly because it seemed desirable to strengthen, if possible, by demonstration on a second species of animal, the assumption that the vascular reactions described by Schlayer are common to all animals. Another point of interest is the attempt to correlate with the anatomical alterations and physiological disturbances in the kidney, changes in the elimination of nitrogen and chlorides in the urine. Studies have also been made of the relation between the gastro-intestinal disturbances and the excretion of nitrogen in the feces and of the relation of such disturbances to anuria.

The lesions produced in the present investigation are those of the better known forms of experimental nephritides, due to potassium chromate, corrosive sublimate, uranium nitrate, arsenic and cantharidin.[3]

As the investigation is modelled largely on that of Schlayer and his associates, their methods and results may be briefly presented.[4]

Schlayer assumed that the vascular reactions of glomerular nephritis should differ from those of tubular nephritis and that with the aid of the oncometer and by the study of diuresis this difference should be readily determined by studying the response of the renal vessels to various stimuli.

To test the power of the vessels to contract, they used sensory stimulation (tobacco smoke in the nose or transient suffocation) as an example of effect through the vasomotor center, and adrenalin as an example of the effect of peripheral contraction. Each of these methods produced a transient contraction of kidney volume with, at the same time, an increase in general blood pressure. Caffein and strong salt solution were used for the purpose of producing dilatation of the renal vessels. With these changes were correlated the variations in the flow of urine.

Potassium chromate and corrosive sublimate were used for the production of tubular nephritis and arsenic, cantharidin and diphtheria toxin for vascular nephritis.

Their opinion concerning tubular nephritis is based on twenty-one experiments with potassium chromate and fifteen with corrosive sublimate. The reactions to the various stimuli in the early stages of this type of nephritis did not differ markedly from the normal. It was found that the animals eliminated a larger amount of urine than do normal animals, which is in accord with

[3] A study of the forms of nephritis due to bacterial poisons, snake venom and similar bodies, has been carried on simultaneously, but will appear as a separate communication.

[4] For a more detailed account see also Pearce, R. M., The Problems of Experimental Nephritis, *The Archives of Internal Med.*, 1910, v.

Weber's[5] observations, and also that diuretics led to a still greater flow, as had also been previously demonstrated by Hellin and Spiro.[6] The vascular reactions differed from the normal only in degree; the power of the renal vessels to contract after sensory stimuli and adrenalin was slightly increased and their power to dilate was increased also to about the same extent.

The results with corrosive sublimate were similar except that the polyuria before the administration of diuretics was not so marked. In both forms epithelial lesions were very prominent, but no anatomical changes were evident in the glomeruli. In short, they consider that the early stages of a tubular nephritis with well marked albuminuria, abundant cast excretion and severe anatomical changes in the tubular epithelium offer no physiological or anatomical evidence of vascular injury.

This, however, was not true of the late stages, which, as well as the more severe forms of tubular nephritis, they found to occupy a middle position between early tubular and typical vascular nephritis. The reactions to sensory stimulus and adrenalin remain practically normal, but the power of dilatation decreases gradually as does also diuresis, and in severe late stages, that is after two to four days, dilatation is very slight or absent, and diuresis does not occur. These changes may be accompanied by slight histological alterations in the glomeruli, but Schlayer and Hedinger believe the condition to be essentially a functional disturbance. That this secondary, glomerular involvement is a true vessel injury and not the result of compression of the glomeruli caused by the retention of urine in tubules blocked by casts, they have shown by experiments in which the ureters were ligated. Under such conditions no vascular disturbances resulted.

In their study of vascular nephritis they used cantharidin and arsenic. In the nephritides thus produced vascular disturbances came on very quickly and in that form due to cantharidin, the early polyuria characteristic of the chromate lesion, was absent. Within four to eight hours the effects of sensory stimulus and adrenalin were much less than in the normal and, after the administration of diuretics, the power of the vessels to dilate decreased, as did also the flow of urine. As the nephritis proceeded to severer degree, or if larger doses of the irritant were given, the power of contraction after sensory stimulus and adrenalin became minimal and dilatation and diuresis became slight or ceased.

The lesions due to arsenic were similar to those of cantharidin except that the general blood pressure fell more quickly and remained at a lower level. This they explained as the result of a greater peripheral capillary injury or, perhaps, a more intense action on the vasomotor center.

In short, Schlayer and his associates have shown that a tubular nephritis, due to chromium or corrosive sublimate with extensive epithelial destruction and a urine rich in albumin and casts, gives

[5] Weber, S., Experimentelle Untersuchungen zur Physiologie und Pathologie der Nierenfunktionen, *Arch. f. exper. Path. u. Pharm.*, 1905, liv, 1.

[6] Hellin, D., and Spiro, K., Ueber Diurese. Die Wirkung von Coffeïn und phloridzin bei artificieller Nephritis, *Arch. f. exper. Path. u. Pharm.*, 1897, xxxviii, 368.

no physiological evidence of vascular disturbance except an increased flow of urine and a slightly heightened response to vascular stimuli. On the other hand, in a glomerular nephritis due to arsenic or cantharidin, with little or no evidence of anatomical injury to either tubules or glomeruli, the vascular reactions are minimal and the disturbance of diuresis approaches anuria. Moreover, the reactions in late tubular lesions approach those of the vascular type.

I. THE VASCULAR REACTIONS.

Methods.—In order to obtain as uniform conditions as possible, animals weighing five to eight kilograms and in good physical condition were selected. These were under observation for several days before injection and the urine was carefully examined for albumin and casts as evidence of spontaneous nephritis.

Physiological studies of each type of nephritis were made on dogs during the first twenty-four hours, and on dogs representing each day of nephritis for five or six days. These included a simultaneous kymographic record of the blood pressure, the kidney volume and the flow of urine. The blood pressure was taken from the left femoral artery with a mercury manometer. The oncometer used for determining the changes in kidney volume was a gutta-percha capsule[7] covered by a plate of glass sealed with a paraffin-vaseline mixture. This was connected with a bellows recorder which responded to the slightest changes in volume and gave both pulse and respiratory waves. The flow of urine from a cannula in the bladder was registered by the usual drop method.

For testing the power of contraction of the renal vessels, adrenalin (Parke, Davis & Co.) in doses of one drop of the 1 to 1,000 solution was used, and for testing the power of dilatation and diuresis, caffein or sodium chloride or both. The caffein was used in the proportion of two cubic centimeters of a one per cent. solution, and the sodium chloride in the proportion of five cubic centi-

[7] This apparatus was made in the laboratory from a model kindly furnished by Dr. C. G. L. Wolf, of the Cornell Medical School, and is a modification of the gutta-percha spleen box first described by Schäfer in his study of the contractibility of the spleen (see Schäfer, E. A., and Moore, B., *Jour. of Physiol.*, 1896, xx, 1). To Dr. Wolf we are also indebted for a model of the bellows recording apparatus used therewith.

meters of a five per cent. solution, per kilo of body weight. Dog's urine was used in doses of three cubic centimeters for the purpose of rapidily lowering blood pressure and also of testing the efficiency of the oncometer. All these fluids were introduced through a cannula in the right femoral vein with due regard to the temperature of the fluids and the speed of injection.

In the earlier and larger part of the experiments, anesthesia was produced by morphin and chloretone;[8] in many of the later experiments Gréhant's[9] method of anesthesia was used, as was also ether.

With the changes determined by physiological and chemical study was correlated, eventually, a study of the anatomical changes. The earlier observations of one of us[10] concerning the disappearance of the depressor substance of the urine in certain stages of tubular nephritis were also continued.

As the result of experiments on nine normal dogs, it was found that adrenalin causes, through its action on the peripheral blood vessels, a decrease in kidney volume and a corresponding increase in blood pressure. Dog's urine causes a marked fall of blood pressure with a corresponding decrease in kidney volume. Both of these are accompanied by a temporary cessation of urine flow. Caffein brings about an increased volume of the kidney accompanied by augmented diuresis. The effect of five per cent. sodium chloride on the kidney vessels is the same as that due to caffein, but caffein increases the general blood pressure, while salt solution causes little or no change, though not infrequently a disturbing temporary fall of pressure occurs.

[8] Morphin, 0.3 c.c. of a one per cent. solution, and chloretone; 10 c.c. of a saturated aqueous solution per kilo of body weight.

[9] Gréhant's anesthesia, as recommended by Sollman, 0.01 gm. per kilo of morphin subcutaneously; after one-half hour, 10 c.c. per kilo of the mixture (chloroform 50 c.c., alcohol and water each 500 c.c.) diluted to 200 c.c. with water and administered by stomach tube. Anesthesia complete in 15 to 20 minutes, lasts several hours. (Gréhant, N., Anesthésie complète produite chez les animaux par le voie sous-cutanée et par la voie stomacale, *Bull. de l'Acad. de méd.*, 1905, liv, 52. See also Sollman, T., Textbook of Pharmacology, Philadelphia, 1906.)

[10] Pearce, R. M., Concerning the Depressor Substance of Dog's Urine and its Disappearance in Certain Forms of Experimental Acute Nephritis, *Jour. of Exper. Med.*, 1910, xii, 128.

The effects of these agents on normal dogs may be seen in Tables I and II and Fig. 1 (Plate XIV).

TABLE I.

NORMAL DOG.

Dog 4, Weight 7,980 Grams, Morphin and Chloretone Anesthesia.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.[11]	Urine: drops in 5 minutes.[12]
Initial	10 : 48	88	0	8
Adrenalin	10 : 53	114	—25	—
	10 : 54–11 : 08	84–80	— 8	5
Caffein	11 : 08–11 : 18	110	+48	55
	11 : 57–12 : 02	130	0	20
Dog's urine	12 : 10	70	—25	—

TABLE II.

NORMAL DOG.

Dog 60, Weight 7,700 Grams, Gréhant's Anesthesia.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	12 : 34	104	0	10
Adrenalin	12 : 39	130	--21	—
	12 : 40–12 : 45	98–104	0	14
Salt solution	12 : 46– 1 : 01	120	+43	35

Reactions in Nephritis.—For convenience of description the early stages of those forms of nephritis due to the action of metallic salts, as potassium chromate, uranium nitrate and corrosive sublimate will be first discussed as examples of early "tubular" nephritis, and for the sake of sharp contrast the so-called vascular forms due to arsenic and cantharidin will then be described. After this comparison the late "tubular" forms which are not readily distinguished from the vascular forms will be presented.

Early Tubular Nephritis.

For the production of this type, potassium chromate and uranium nitrate have been given in single doses of 0.06 gram and 0.015

[11] The horizontal level of the writing pen at the beginning of the experiment is taken as normal kidney volume for the conditions obtaining. Increase or decrease in volume, as the case may be, is indicated in millimeters preceded by a plus or minus sign. The change in level was measured on the tracing with the base line as a constant factor.

[12] Drops from bladder cannula as registered on drum.

gram, respectively, and corrosive sublimate in doses of 0.02 gram daily. As a rule, these doses have produced severe lesions without causing early death of the animals.

Chromate Nephritis.—Four dogs have been examined during the first twenty-four-hour period following injection. The vascular reactions in this stage do not differ from those in the normal animal except that the power of the vessels to contract may be somewhat exaggerated. Thus, the fall in kidney volume after adrenalin is usually extreme, as is also that due to dog's urine. The power of dilatation after caffein or salt solution in this stage is equal to or greater than that of the normal kidney and is accompanied by a diuresis of corresponding degree.[13]

This early state is illustrated by the following table:

TABLE III.

CHROMATE NEPHRITIS.

Dog 62, Weight 5,480 Grams, Gréhant's Anesthesia. Received 0.03 Gram of Potassium Chromate Subcutaneously, 20 Hours Before Testing. Urine Contained 1 Gram of Albumin by Esbach and Numerous Casts at Time of Test.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	11 : 26–11 : 31	74	0	4
Adrenalin	11 : 31	100	—21	—
	11 : 33	76	0	—
Caffein	11 : 34			
	11 . 44	104	+12	60
Salt solution	11 : 56–11 : 66	110	+30	140
Dog's urine	11 : 67	44	+16	—

Histological examination of this kidney showed the epithelium of the convoluted tubules to be swollen and granular, with numerous small areas of complete necrosis involving one, two or three tubule loops. The cells of the loops of Henle showed many vacuoles. Numerous hyaline and coarse granular casts occurred in the collecting tubules. The medulla of the kidney was slightly edematous and throughout the section was seen evidence of slight leucocytic infiltration. The veins of all sizes were congested as were also the capillaries of the glomeruli; no anatomical changes in the glomeruli were seen.

[13] An exception to this rule was observed in one animal with a twenty-one-hour nephritis. Caffein caused abundant diuresis with a flow of 75 drops of urine per five minutes, but *without increase in the kidney volume.* The administration later of 5 per cent. sodium chloride increased the diuresis to 105 drops of urine per five minutes without change in kidney volume. This is the only observation in this series of experiments of diuresis produced by caffein or salt without a corresponding increase in kidney volume.

After the first day the character of the reactions in chromate nephritis varies. As will be shown in the discussion of the late stages, the progress of a lesion viewed merely from point of time elapsed is relatively unimportant. The main point is the severity of the lesion. Thus, although the late lesions approach the vascular type, it is possible to have a lesion of the fourth day, the reaction of which corresponds to those of the first day or even approaches in character the reactions of the normal dog. This is shown in the following experiment:

Dog 63, weight 9,320 gm. Received on November 19, 0.06 gm. potassium chromate subcutaneously. On November 20, the urine contained casts and 2.5 gm. albumin per liter and on the 21st 1.75 gm. per liter. On November 23, the animal was used for physiological study. The urine of the past twenty-four hours amounted to 755 c.c., had a specific gravity of 1,008, contained numerous casts and .25 gm. of albumin by Esbach. The animal was in good condition; no vomiting or diarrhea; did not refuse food. The depressor substance did not disappear from the urine. (Gréhant's anesthesia.)

TABLE IV.

CHROMATE NEPHRITIS.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	11 : 10–11 : 15	98	0	7
Adrenalin	11 : 15	130	—24	—
	11 : 21	98	0	—
Caffein	11 : 22			
	11 : 29–11 : 34	112	+21	29
	11 : 39–11 : 44	116	+32	60
Salt solution	11 : 50			
	11 : 59–12 : 04	110	+39	119
Dog's urine	12 : 05	76	+28	—

Uranium Nephritis.—The early stage of this form differs in no way from that due to potassium chromate. Nephritis of the first and second day shows adequate vascular reactions and efficient diuresis.

The following experiment is illustrative.

Dog 22, weighing 5,820 gm., received 0.015 gm. of uranium nitrate subcutaneously at 3 P. M. on May 8. Physiological observations were begun on May 10 at 10:30 A. M., 43 hours after injection. Morphin and chloretone anesthesia was used. The urine of May 10, representing the preceding twenty-four hours, amounted to 185 c.c., with a specific gravity of 1,021, and contained an abundance of albumin.

TABLE V.

URANIUM NEPHRITIS.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	10 : 31–10 : 36	76	0	8
Adrenalin	10 : 36	120	—15	—
	10 : 40–10 : 45	70	0	10
Caffein	10 : 45			
	10 : 55	88	+20	46
	11 : 25–11 : 30	94	+38	98
	11 : 36–11 : 41	76	+38	35
Salt solution	11 : 41–11 : 56	76	+47	65

Histological examination showed extensive degeneration of tubular epithelium, but no glomerular lesion.

Corrosive Sublimate Nephritis.—The reaction in the early stages of this form agree in every respect with those of like stages of the chromate and uranium lesions except that they may persist until the third day.

The following experiment is illustrative.

Dog 42, weighing 4,240 gm., received on June 4, 5 and 6, subcutaneously, 0.02 gm. of corrosive sublimate. The urine of June 7 amounted to 630 c.c., had a specific gravity of 1,007, and contained a small amount of albumin and a few casts. (On June 3, the amount of urine was 125 and the specific gravity 1,020.) On June 7, under morphin and chloretone anesthesia, physiological tests were made.

TABLE VI.

SUBLIMATE NEPHRITIS.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	10 : 30–10 : 35	76	0	10
Adrenalin	10 : 36	126	—45	—
	10 : 38–10 : 41	76	0	9
Caffein	10 : 41			
	10 : 52–10 : 57	96	+36	65
	11 : 02–11 : 07	108	+45	80
Dog's urine	11 : 08	58	— 2	—

Histological examination showed degeneration of the convoluted tubules; no calcification of the loops of Henle and no changes in the glomeruli were seen.

The characteristics of this early stage of tubular injury may be briefly summarized as follows: the animals, irrespective of the nature of the irritant, present usually an initial polyuria; the vas-

cular reactions are normal or exaggerated and, with the increased dilatation due to the administration of caffein and salt solution, there occurs a correspondingly greater diuresis. In chromate nephritis this stage has not been seen after the first day, except in one animal of the fourth day; in uranium nephritis it has continued to the second day, and in sublimate nephritis as late as the third day. These results for the dog agree with those of Schlayer for the rabbit and may be considered as characteristic of the early lesion due to a poison causing injury to the tubular epithelium with no anatomical evidence of vascular injury. But, despite the absence of anatomical lesions in the glomeruli, the exaggerated vascular reactions and increased diuresis point to a functional disturbance of the vessels, presumably an increased excitability. As will be shown later in the discussion of typical vascular nephritis, a similar condition may occur after the use of arsenic and cantharidin in doses insufficient to cause vascular incompetency. It is possible, therefore, that as vascular poisons may, in small doses, lead by mild irritation to changes characterized by increased vascular activity, so the poisons classified as tubular may have also an influence on the vessels and as the result of continued irritation lead to the relative renal incompetency to be discussed under the head of late tubular lesions. To test this view, experiments have been made on several normal animals by injecting epithelial poisons after the normal flow of urine has been determined. The occurrence of an increased flow of urine under such circumstances has been considered as evidence of increased vascular excitability occurring independently of tubular injury. Thus, it has been found that two minutes after the subcutaneous injection into a normal dog of 0.12 gram of potassium chromate, an increased flow of urine occurs at the rate of twenty-six drops per five minutes as compared with the previous flow of six drops per five minutes. This occurs without a rise in blood pressure of more than four millimeters of mercury, while at the same time the kidney volume gradually sinks and with this fall, which may be as much as twenty-three millimeters in half an hour, diuresis gradually lessens but does not fall below the normal. The power of the kidney vessels to contract under the influence of adrenalin is not greater than in the normal period.

Uranium nitrate administered under similar conditions causes only a slight increase in urine flow; six drops per five minutes as compared with a previous rate of four per five minutes; at the same time the kidney volume decreases ten millimeters in five minutes, despite a slight rise in blood pressure of four millimeters of mercury. At the end of half an hour no further change occurs. It is evident, therefore, that so-called tubular poisons have an immediate effect on the secreting power of the kidney, which is of the nature of an increased excitability and presumably is independent of the tubular injury. It is not possible, therefore, to consider the phenomena of this stage to be entirely independent of an effect of the poison on the glomerulus. To this we will return in the discussion of late tubular lesions.

Vascular Nephritis.

Arsenic and cantharidin have been used to produce the forms of experimental nephritis commonly described as vascular.

Arsenic Nephritis.—The immediate effect of arsenic, administered as arsenious acid, is shown in the following experiment in which a normal animal properly prepared for physiological tests was given arsenic intravenously and the reactions studied as soon as it was evident that this substance had affected the blood vessels.

TABLE VII.

ARSENIC NEPHRITIS.

Dog 75, Weight 11,230 Grams, Ether Anesthesia.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	10 : 52–11 : 21	102	0	4
Arsenious acid 0.06 gm. in vein	11 : 21–11 : 29	84	—17	6 drops in 8 min.
Caffein	11 : 29–11 : 44	100	from —17 to —10	1 drop in 15 min.
Salt solution	11 : 44–11 : 59	100	from —10 to + 8	7
Dog's urine	12 : 05	58	from + 8 to —17	—
Adrenalin	12 : 15	116	from — 6 to —10	—

The urine from the bladder, at the time of inserting the cannula, contained no albumin; at 12:35 the small amount which was collected showed definite coagulum

by heat and acetic acid test, and that in the cannula at the end of the experiment (12:54) showed a still greater coagulum. Histologically the glomeruli were found to be congested and to fill completely the capsular space. The tubular epithelium was swollen, more or less granular and somewhat vacuolated.

The fall in blood pressure and in kidney volume illustrates the paralyzing effect of arsenic on the capillaries; the presence of albumin in the urine indicates an alteration in the permeability of the capillary walls. The administration of caffein and salt solution is accompanied in the case of the former by a minimal dilatation and in that of the latter by only a moderate dilatation. (See Plate XVI, Fig. 3.) Efficient diuresis is not produced by caffein and but little more than the normal flow by salt solution. The vascular reactions caused by adrenalin and dog's urine are very slight; in the case of the former the fall in kidney volume is minimal. The return to the former level after the injection of dog's urine is greatly prolonged compared with the quick return in normal animals and in those with tubular lesions. This experiment may be taken as the typical early vascular lesion due to a large dose of arsenic. The results differ from those of Schlayer who found that in the rabbit salt solution caused an increase of diuresis followed later by anuria.

The study of advanced lesions has been difficult on account of the varying susceptibility of the animals to arsenic. Injection of less than 0.03 gram has not, as a rule, caused a serious kidney lesion, but this dose usually kills two-thirds of the animals in twenty-four hours. The following experiment is illustrative of an animal surviving such a dose.

Dog 27, weighing 7,140 gm., received 0.03 gm. of arsenious acid, subcutaneously, on May 17 (3:30 P. M.). On the following day, after an interval of 19 hours, physiological tests were made under morphin and chloretone anesthesia. The animal had severe diarrhea and excessive vomiting. On placing the cannula in the bladder, no urine was found; the cannula and bladder were filled with warm salt solution and during the entire experiment only six drops escaped from the cannula.

Adrenalin caused a moderate contraction of the blood vessels, but a contraction considerably greater than that in the previous experiment. The depressor effect of dog's urine was also greater, but the return to normal level was very slow. Caffein caused an increase in kidney volume of only 11 mm. without flow of urine. The vascular change due to salt solution was obscured by a hemorrhage into the oncometer, but during a period of 55 minutes following this injection only six drops of urine were recorded.

Such are the reactions of a severe vascular nephritis of the first twenty-four hours. All attempts to produce disturbances of severe degree over longer periods of time have been unsuccessful. An amount of arsenic sufficient to produce a serious disturbance of the kidney function always caused early death of the animal; on the other hand, small doses which allow survival of the animal, do not lead to a nephritis with vascular incompetency. They cause rather a type of nephritis closely resembling the early tubular variety. This is illustrated in the following experiment.

Dog 30, weighing 8,480 gm., received daily 0.01 gm. arsenious acid subcutaneously for five days. On the sixth day the vascular reactions were studied under morphin and chloretone anesthesia. The urine had increased from 140 c.c. with a specific gravity of 1,030 on the first day to 455 c.c. with specific gravity of 1,010 on the sixth day; only traces of albumin were present.

TABLE VIII.

ARSENIC NEPHRITIS.

	Time.	Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	11 : 09–11 : 14	100	0	20
Caffein	11 : 14–11 : 19	134	+24	65
	11 : 29–11 : 39	126	+41	110
	11 : 46–11 : 51	116	+53	85
Adrenalin	11 : 52	142	+20	—
	11 : 52–12 : 12	122	+58	80
Dog's urine	12 : 12	60	+ 3	
	12 : 17–12 : 37	114	+60	75

At the end of the experiment the oncometer was free of blood. Histologically the kidneys presented considerable granular degeneration of the convoluted tubules, vacuolization and fatty change in the loops of Henle. The glomeruli showed no anatomical changes.

These results, which have also been seen in a dog of the third day, can only be interpreted as being of the same character as the early lesion due to epithelial poisons. They cannot, especially with the presence of small amounts of albumin, be explained as the effect of stimuli on a normal kidney. (See also Plate XV, Fig. 2.)

One must consider, therefore, whether in such cases arsenic has not an effect on the vessels of the nature of an increased excitability which is responsible for the more ready reaction to stimuli. If this be the case, we are obliged to look upon these manifesta-

tions as well as the similar phenomena of early tubular nephritis as analogous in character and representing an early vascular irritability which, if the excitation is continued or increased, leads to vascular incompetence and complete lack of response to stimuli.

Cantharidin Nephritis.—This form closely resembles that caused by arsenic. The immediate effect of cantharidin on the renal blood vessels of the normal dog is shown in the following experiment.

Dog 77, weighing 6,280 gm., received, under ether anesthesia, 3 c.c. (0.04 gm.) of a saturated solution of cantharidin in acetic ether *intramuscularly.* Within one minute the kidney volume began slowly to fall and after six minutes the recorder reached a level 47 mm. below the initial reading.[14] At the same time the blood pressure, originally 106 mm. of mercury, fell slowly to 90, and then rose rapidly to 132 mm. This, its highest point, at the end of five minutes, corresponded to the most rapid fall in kidney volume (36 mm.). During the sixth minute the blood pressure fell rapidly, almost to the abscissa, and death resulted.

A less profound immediate effect after subcutaneous injection is shown in the following experiment.

TABLE IX.

CANTHARIDIN NEPHRITIS.

Dog 79, Weighing 9,640 Grams, Under Ether Anesthesia.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	11 : 10–11 : 25	84	0	5
0.053 gm. cantharidin subcutaneously	11 : 27			
	11 : 33	92	—33	3 in 6 minutes.
Adrenalin	11 : 33	108	—26	—
Dog's urine	11 : 35	88	From —32 to —26	—
Caffein	11 : 39			
	11 : 47	84	—25	0 in 14 minutes.
Salt solution	11 : 47–12 : 00	92	—19	2 in 13 minutes.
Salt solution repeated	12 : 01–12 : 41	96	0	5
	12 : 41–12 : 51	84	— 5	1
	12 : 51– 1 : 01	74	— 5	1 in 10 minutes.

In this experiment the greatly diminished power of the vessels to react to stimuli is well shown, as is also the development of a

[14] This experiment, as well as that which follows, has been controlled by the injection of acetic ether with negative results except for a slight rise in blood pressure (5 mm.) which is not accompanied by alteration in kidney volume or flow of urine.

temporary anuria. Later, after caffein and two injections of salt solution, the kidney volume returned to the initial level and a diuresis equal to that at the beginning of the experiment was obtained. Soon, however, the condition of anuria again developed. The urine collected after the injection of cantharidin contained a considerable amount of coagulable protein.

The later stages of cantharidin nephritis, resulting from the daily injection of small doses (.013 gram), represent a gradually developing renal incompetency, which may be considered as characteristic of severe vascular nephritis. Such conditions have been found on the third and fifth day. One of these experiments follows.

Dog 37, weighing 7,320 gm., received daily 0.013 gm. cantharidin in acetic ether, subcutaneously. The urine increased on the second day from 140 to 520 c.c. and then diminished rapidly to only 86 c.c. on the fifth day; the specific gravity rose from 1,012 on the second day to 1,056 on the fifth. Albumin increased from a trace to 2.5 gm. per liter by Esbach on the fourth day; on the fifth day, when the urine was blood stained, it reached 5 gm. per liter. The sediment contained red blood corpuscles, epithelial cells and hyaline and granular casts. Vomiting was a disturbing factor and diarrhea began on the fifth day. Physiological observations were made on the latter day under morphin and chloretone anesthesia. On inserting the bladder cannula, one and a quarter hours after catheterization, no urine was found.

TABLE X.

CANTHARIDIN NEPHRITIS.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops.
Initial	10 : 15–10 : 20	50	0	0
Caffein	10 : 20–10 : 25	54	+ 2	0
Salt solution	10 : 26–10 : 46	66	+16	0
Adrenalin	10 : 47	98	from +16 to −41	0
Final readings	11 : 07	35	+ 2	0

At the autopsy no hemorrhage was found in the oncometer and the ureters were not obstructed. Upon histological examination the cells of the convoluted tubules were swollen, and by Sudan III showed considerable fatty transformation; in many cells definite mitotic figures were seen. The convoluted tubules, but more especially the loops of Henle contained many large pale cells with nuclear fragments. These lay free in the lumen. The glomeruli were congested, but otherwise were normal in appearance. The cell accumulations in Bowman's capsule usually described were not present.

The above experiment is not entirely satisfactory in that the low blood pressure is, in itself, a sufficient explanation of the absence of urine. That low pressure, however, is not the only factor is shown in another observation on an animal of the third day with an initial blood pressure of 86 millimeters of mercury. In this animal the kidney volume could not be raised to more than + 13 by caffein and salt solution and during a period of an hour and a quarter only fourteen drops of urine were excreted.

These results are in close accord with those obtained in severe arsenic nephritis, and also with the anuric type of late chromate nephritis to be described later, and may be considered as evidence of vascular incompetency. However, cantharidin, under certain circumstances may, like arsenic, produce a condition not to be distinguished from the early tubular lesion. This was seen once in this series in an animal examined nineteen hours after a single injection of 0.013 gram of cantharidin. The urine contained albumin, numerous red blood corpuscles, a few epithelial cells, but no casts. The general condition of the animal was good. Under morphin and chloretone anesthesia, exaggerated vascular reactions were obtained, the kidney recorder registering — 53 after adrenalin and + 28 after salt solution injection with a maximum flow of urine of 75 drops per minute.

It is evident that this latter condition, which occurs in both arsenic and cantharidin poisoning after small doses or short periods of time, must be considered as a stage of increased irritability of the vessels preceding the incompetency which follows larger doses or long continued action. If this view is accepted we must then consider the early polyuric stage of chromate and uranium to be also a manifestation of increased vessel irritability independent of the tubular lesion. Such a view is opposed to the conception of exclusively vascular and exclusively epithelial lesions, but not to the view supported by general clinical and pathological observations, that either of these types of lesions may be the predominating characteristic of a nephritis.

Late Tubular Nephritis.

This stage of chromate, uranium and sublimate nephritis differs from the early tubular type as well as from the typical vascular

nephritis in two prominent characteristics. The first of these is the diminished power of the vessels to dilate after the administration of caffein and salt solution, which decreased dilatation, however, is seldom as slight as that of vascular nephritis. The second characteristic is the frequent complete or almost complete failure of the stimuli to cause diuresis, irrespective of the degree of dilatation[15] of the vessels. These characteristics allow us to differentiate between two varieties of late tubular nephritis. In one variety occurs severe gastro-intestinal disturbances and diminished secretion of urine; in the other, gastro-intestinal disturbances are not prominent, and well marked polyuria is present up to the moment of anesthesia. The first of these represents apparently a nephritis of a character so severe that the animal presumably cannot recover.

This is illustrated in the following experiment.

Dog 38, weighing 5,540 gm., received on May 27, 0.06 gm. of potassium chromate. The urine of May 28 amounted to 300 c.c. with a specific gravity of 1,017, and contained casts and albumin. Vomiting began on this day and continued more or less constantly to the end. On May 29, the total amount of urine was 174 c.c.; on the thirtieth it was 120 c.c.; on June 1 and 2, 28 and 25 c.c. respectively. The urine of each day contained albumin, finely granular casts in great numbers, a few white blood cells and many epithelial cells. On the fifth day of nephritis physiological tests were made under morphin and chloretone anesthesia, as shown in the following table. On opening the bladder no urine was found.

TABLE XI.

CHROMATE NEPHRITIS.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	3 : 29–3 : 34	86	0	0
Caffein	3 : 34			
	3 : 34–3 : 39	104	+ 8	0
Salt solution	3 : 39–4 : 14	112	+23	None in 35 minutes.
Adrenalin	4 : 15	150	—22	—

At the completion of the experiment the oncometer was free of blood and no obstruction of the ureters could be demonstrated. No urine was found in the pelvis of the kidney; the kidney itself was congested and edematous. Histologically, diffuse necrosis of convoluted tubules was found, and by Sudan III, extensive fatty transformation involving all tubules, most marked in the loops

[15] Every precaution in the way of control experiments has been taken against error due to hemorrhage into the oncometer, the influence of external stimuli, operative injury and ureteral obstruction. None of these can be considered as a factor in the results described in the experiments here presented.

of Henle. The glomeruli were somewhat contracted, but showed no changes except an ill-defined thickening of the capillary loops which rendered their outlines indistinct. All tubules contained an abundance of casts.

The following experiment illustrates an almost complete anuria in a second day nephritis without prominent gastro-intestinal disturbances.

Dog 16, weighing 8,440 gm., received 0.06 gm. of potassium chromate on April 3. The urine of April 4 contained casts and 5 gm. of albumin per liter by Esbach's method; severe vomiting began on April 5, and on this, the second day of nephritis, 43 hours after injection, physiological tests were made under morphin and chloretone anesthesia.

TABLE XII.

CHROMATE NEPHRITIS.

Time.		Blood pressure in mm. Hg.	Kidney volume in mm.	Urine: drops in 5 minutes.
Initial	10 : 58–11 : 03	82	0	2
Adrenalin	11 : 03	108	46	—
	11 : 16	80	0	1 drop in 13 minutes.
Caffein	11 : 17			
	11 : 37	74	+16	2 drops in 20 minutes.
	12 : 05	60	+11	4 drops in 27 minutes.
Salt solution	12 : 06			
	12 : 21	68	+28	9 drops in 15 minutes.

The examination of bladder and ureters at the end of the experiment showed no evidence of obstruction to flow of urine. The histology of the kidney is similar to that of animal of Table XI.

These experiments illustrate late tubular lesions characterized by a degree of vascular incompetency sufficient to justify the conclusion that this stage may be considered as approaching the vascular type of nephritis. The increase of kidney volume, due to caffein, is minimal and that due to salt solution far below the normal; by neither is efficient diuresis produced. (See Plate XVII, Fig. 4, and compare with Tables XI and XII.) The power of contraction of the vessels on the other hand is not diminished to as great an extent as in true vascular nephritis due to arsenic or cantharidin.

These results are closely analogous to those which Schlayer describes as characteristic of the late stage of tubular nephritis in the rabbit, and represent what we have termed the anuric type.

The anuric type of late tubular nephritis is not, however, the common lesion. It is apparently limited to those animals which present the most severe symptoms and which succumb, or if not used for the physiological experiment presumably would eventually have succumbed to the experimental disease. Most of the animals, in all three varieties of epithelial nephritis (chromate, uranium and sublimate), pass rapidly into a condition characterized by polyuria (hyposthenuria) with more or less general weakness and little or no gastro-intestinal disturbance. The amount of urine eliminated is very great, 600 to 1,200 cubic centimeters in twenty-four hours, with a specific gravity varying from 1,008 to 1,020, as compared with the normal specific gravity of 1,035 to 1,050. This form, which for convenience of description we will refer to as the polyuric type, presents this peculiar feature; it matters not how great the polyuria may be, this ceases as soon as the animal receives an anesthetic, and instead of polyuria, a condition of complete or almost complete suppression develops. This suppression moreover is not due to the failure of dilatation of the renal vessels.

This is seen in Dog 17, in the third day of nephritis. After the injection of caffein, the kidney volume rose to + 33, an increase equal to that of a normal dog, and which should, theoretically, be accompanied by a profound diuresis, whereas during a period of forty minutes following the injection only nineteen drops of urine were passed. In this and similar experiments no mechanical obstruction of the ureters could be found at the end of the experiment. In one of the animals, under chloroform anesthesia, the general blood pressure was so low (58 at the beginning of the experiment and falling gradually to 46 millimeters of mercury) that the absence of urine flow could thus be explained. This, however, was not the case with other animals for with ether anesthesia and a blood pressure of 120 millimeters of mercury diuresis has failed also. We are forced, therefore, to the conclusion that the so-called late tubular nephritis with polyuria, is accompanied by a condition of the vessels which allows dilatation but is so influenced by an anesthetic as to cause decreased glomerular permeability. That the anesthetic, and not the operative procedure,

is responsible for this change has been demonstrated in carefully conducted control experiments.[16]

This peculiar reaction of the polyuric type of late tubular nephritis resembles somewhat the intermediate stage which Schlayer and his associates describe as characteristic of uranium nephritis in the rabbit. Uranium nephritis, they classify as a tubular nephritis, but describe an intermediate stage during which the administration of five per cent. sodium chloride solution causes extreme dilatation with strong pulsation but no corresponding diuresis. This stage develops thirty-six to forty-eight hours after the onset of the experimental disease, at a time when no edema is evident but when the urine is decreased in amount as compared with the preliminary polyuria. The vessels react to contraction stimuli strongly, the blood pressure shows no change, the power of dilatation of the vessels is retained but is unaccompanied by flow of urine. This occurrence, which they observed in fourteen animals, would appear to offer definite evidence of decreased permeability of the glomerular vessels, marking possibly, in their opinion, a pre-edemic stage. They also found that some minutes after the inhibition of diuresis, caused by the salt injection, a few drops of urine are excreted, but no further improvement in the flow of urine occurs. If after a lapse of twenty minutes or so, they injected caffein, the kidney volume which had fallen, increased to the maximum attained after the previous salt injection, and a slight or moderate flow of urine occurred.

That the peculiar phenomenon of dilatation without diuresis, which we have seen, is similar in many ways to that described by Schlayer as characteristic of uranium nephritis in the rabbit, is evident, but that it is not entirely analogous is shown by the following observations: (1) it is preceded until the moment of anesthesia by polyuria; (2) after anesthesia, anuria is frequently complete; (3) in many instances no diuresis can be induced by caffein, and (4) it occurs in chromate as well as in uranium nephritis.

[16] The details of these experiments, with special reference to the phenomena of the polyuric type and the influence of various anesthetics will be presented in a later communication. The brief description here given is introduced merely for the purpose of presenting a complete description of the vascular reactions in all types of nephritis.

We do not wish to be understood as implying that Schlayer's intermediate stage of uranium nephritis is to be explained by the influence of an anesthetic, for we have not studied nephritis in the rabbit and have no knowledge of the influence of urethane, the anesthetic used by Schlayer, upon renal function. In our experiments, however, there is no doubt that the results are to be so explained.

II. THE ELIMINATION OF NITROGEN.

The chemical studies have had for their object the determination, if possible, of changes characteristic of tubular as contrasted with vascular nephritis, and have been limited to the study of the total nitrogen and total chloride elimination. In view of the generally accepted theory that most, if not all, of the urinary nitrogen is eliminated by the tubular epithelium, it was assumed that in severe tubular lesions a decrease in the nitrogen output would be observed. As an index of salt elimination, the chlorides were selected for study because of their importance in the pathology of nephritis. The dogs used in this phase of the work were placed on a diet of beef heart, lard and cracker until a condition of nitrogen equilibrium was reached and nephritis was then produced by various irritants. In most of the experiments the animals received a measured amount of water by stomach tube daily; the estimations were made on exact twenty-four hour amounts from catheterized animals. The Kjeldahl method was used for nitrogen determination and the modified Volhard method for chlorides.

The results with certain forms of nephritis, on account of the early appearance of gastro-intestinal disturbances, have not been very satisfactory. This is particularly true of chromate nephritis in which vomiting frequently occurs shortly after the production of the experimental lesion and either persists or continues more or less intermittently. The inability to retain food necessarily prevents a study of nitrogen elimination based on a previous nitrogen equilibrium, and as the difficulties of determining, under such circumstances, the percentage of nitrogen ingested are very great, such experiments have been abandoned on the appearance of digestive disturbances. With uranium nitrate and arsenic nephritis, however, sufficiently satisfactory results have been obtained to warrant

tentative conclusions concerning the elimination of nitrogen in tubular and glomerular nephritis respectively. In some of the tables the nitrogen of the feces is also given. The latter estimations were made for the purpose of determining, if possible, whether or not the gastro-intestinal disturbance was the result of a vicarious elimination of nitrogenous substances through the alimentary canal. One[17] of us has elsewhere tested this hypothesis in animals with kidney reduction, a condition likewise accompanied by digestive disturbances, but with negative results.

Table XIII illustrates the difficulties of metabolism studies of chromate nephritis. Vomiting occurred on the day of injection and digestive disturbances continued to the end of the experiment, which, however, was continued for the purpose of determining the elimination of fecal nitrogen.

TABLE XIII.

Dog 4—Potassium Chromate.

Date. 1909		Weight in gm.	Urine.					Feces.		Notes.
			Amount in c.c.	Spec. grav.	Total N in gm.	Alb. N in gm.	Total chlorides in gm.	Total N in gm.	Per cent. N.	
Feb.	16	6860	440	1015	—	—	—	0.2678	5.73	
	17	6855	430	1015	5.72	0	1.4	0.3645	5.87	
	18	6780	140	1050	5.79	—	1	0.2579	6.25	Control period: no albumin,
	19	6740	120	1045	5.81	0	1.1	0.2579	6.25	no casts.
(1)	20	6520	160	1036	5.64	—	—	0.2165	6.06	Received 0.06 gm. potass. chrom. subcutaneously.
	21	6360	470	1026	5.44	0.481	0.5	0.2165	6.06	Casts + + +, vomiting.
	22	6200	390	1022	2.40	0.118	0.7	0.2165	6.06	Gastro-intestinal disturbances.
	23	6100	280	1015	1.94	—	0.3	0.1426	7.51	" "
	24	6000	270	1010	2.07	—	0.12	0.1426	7.51	" "
	25	5810	345	1011	2.42	0.035	0.4	0.1426	7.51	" "
	26	5700	565	1011	2.77	0.025	0.5	0.1426	7.51	" "
	27	5420	385	1012	3.06	0.134	0.3	0.2604	4.21	5 gm. sodium chlorid, given by stomach.
	28	5200	635	—	4.43	0.074	1.3	0.2604	4.21	Vomiting and diarrhœa excessive.
March	1									Died in coma.

Two other dogs with chromate nephritis gave equally unsatisfactory results. A fourth dog received several doses over a longer

[17] Pearce, R. M., The Influence of the Reduction of Kidney Substance upon Nitrogenous Metabolism, *Jour. of Exper. Med.*, 1908, x, 632.

period of time, and by reducing the first dose to 0.03 gram, a fairly satisfactory study of the nitrogen elimination was made.

Dog 51, weighing 10,930 gm., was placed on a constant diet of beef heart, cracker and lard, with bone ash, on October 25. The urine from that date until November 1 was free from albumin and casts.

TABLE XIV.

Dog 51—Potassium Chromate.

	Date.	Urine.			Notes.
		Amount in c.c.	Specific gravity.	Total N in gm.	
	Nov. 2, '09	165	1047	7.28	No albumin; no casts.
	3	160	1046	7.08	" "
	4	135	1054	7.09	" "
(1)	5	130	1059	—	0.03 gm. potass. chrom. subcutaneously.
	6	145	1056	7.18	Casts abundant; large amount albumin.
	7	170	1052	6.69	Slight diarrhœa.
(2)	8	200	1042	7.79	0.03 gm. potass. chrom. subcutaneously.
	9	155	1051	6.24	
	10	340	1027	6.31	Slight diarrhœa.
	11	170	1048	7.72	
(3)	12	150	1050	7.19	0.06 gm. potass. chrom. subcutaneously.
	13	330	1040	6.42	
	14	680	1033	7.04	Diarrhœa; did not eat all food; vomiting.
	15	670	1024	—	" " " " " " "
	16	670	1019	—	Diarrhœa and vomiting ceased; appetite good.
	17	620	1015	7.59	
(4)	18	600	1013	7.02	0.06 gm. potass. chrom. subcutaneously.
	19	300	1024	6.01	
	20	610	1021	6.71	Slight vomiting and diarrhœa; appetite good.
	21	700	1016	7.76	Slight diarrhœa.
	22	350	—	7.29	" "
	23	630	1015	—	Discontinued on account of severe diarrhœa.

In this table the daily average nitrogen elimination for eighteen days of nephritis is seven grams as compared with 7.15 grams for the three days of the normal period, a difference too slight to be of significance. It is of interest, however, that after the second dose of chromate on November 8, a very decided drop in nitrogen excretion occurred for forty-eight hours, and a similar drop for a like period after the fourth injection on November 18. The third injection (Nov. 12) was followed by a drop for one day and the first injection by a like fall on the second day. Although these decreases vary considerably, they equal 6 to 16 per cent. of the normal elimination and doubtless represent the degree of temporary insufficiency of the renal tubules. The results, however, are not

as definite as those of Green[18] who describes a nitrogen decrease of twenty per cent. in chromate nephritis in the dog. It is of importance to note, however, that diarrhea and other gastro-intestinal disturbances, as a rule, follow this decreased elimination of nitrogen, and after their cessation the elimination of nitrogen increases above the normal. As may be seen in Table XIII, the feces show no increased elimination to correspond with the decreased nitrogen of the urine, and the gastro-intestinal symptoms cannot, therefore, be due to the vicarious elimination of toxic nitrogenous bodies. The coincidence of gastro-intestinal disturbance and diminished nitrogen elimination, however, suggests that toxic substances, non-nitrogenous in nature, may be retained at the same time as the nitrogen and that these are responsible for the vomiting and diarrhea. This view is supported also by the observation that on November 13, the only time a test was made for the depressor substance, it was found to be absent; on this day the decrease in nitrogen was 10 per cent. of that eliminated on the previous day.

TABLE XV.

Dog 8—Uranium Nitrate.

Date.	Weight in gm.	Urine.					Feces.		Notes.
1909		Amount in c.c.	Spec. grav.	Total N in gm.	Alb. N in gm.	Total chlorides in gm.	Total N in gm.	Per cent. N.	
March 1	9380	520	1020	7.50	0	0.8	0.3113	2.03	
2	9440	550	1018	7.50	0	0.76	0.3113	2.03	
3	9310	510	1018	7.29	0	0.6	0.3113	2.03	0.015 uranium nit. subcutaneously.
4	9020	505	1017	6.23	0.123	0.64	0.3113	2.03	Casts + + +.
5	9200	605	1022	6.41	0.291	0.36	0.4556	3.19	
6	9065	670	1020	6.43	0.134	0.28	0.3784	3.34	
7	8520	960	1019	6.40	0.134	0.35	0.3784	3.34	
8	8140	745	1018	6.66	0.063	0.32	0.3784	3.34	Vomiting.
9	7800	460	1017	3.24	0.044	0.56	0.3825	3.19	Vomiting and diarrhœa.
10	7600	240	1013	Discontinued.		0.52	0.1787	7.23	Vomiting and diarrhœa; refuses food.
11	6940	190	1012			0.68	0.1787	7.23	500 c.c. normal salt solution in vein.
12	6940	—	—			4.00	0.1787	7.23	
13	6700	260	1006			1.68	—	—	Died 10 a. m. in coma.

[18] Green, J. L., Experimental and Clinical Observations on the Urine and Blood in Nephritis, *Jour. of Path. and Bact.*, 1909, xiii, 296.

The experiments with uranium nephritis are more satisfactory in that the gastro-intestinal symptoms do not come on so rapidly and a definite decrease in urinary nitrogen is demonstrable. This is shown in Table XV.

Dog 8, weighing 9,380 grams, was placed on a constant diet of beef heart, cracker and lard on February 23, and received 500 c.c. of water by stomach tube daily. The urine during the control period was free of albumin and casts.

Here, for a period of five days, the average daily nitrogen is 6.43 grams as compared with the average of 7.43 grams for the normal period, an average decrease of 13.5 per cent. On the fifth day, the gastro-intestinal disturbance began and continued until death on the eleventh day. The examination of the feces, it will be seen, throws no light on the fate of the retained urinary nitrogen or on the cause of the gastro-intestinal disturbance. The percentage increase of fecal nitrogen of the last three days is probably to be explained by coagulable protein due to diarrhea. A decrease or urinary nitrogen in this form of nephritis in the dog has been described also by Siegel[19] who likewise found no increase of fecal nitrogen.

Similar studies of nitrogen elimination in animals with vascular nephritis are best illustrated by the experiment with arsenic as seen in the table below.

Dog 12, weighing 10,500 grams, was placed on constant diet on March 20. During the control period the urine was free of albumin and casts. Throughout the experiment the animal was given 500 c.c. of water by stomach tube daily. Nephritis was induced by arsenious acid on April 3, with injections also on April 5, 8, 10 and 13.

It is seen that in this distinctly vascular lesion, despite occasional vomiting, the power of the kidney to eliminate nitrogen has not suffered. Indeed, this animal showed an increased elimination, equal, on the fourth day, to 14 per cent. of the normal average, and for the entire period of thirteen days, equal to six per cent. of the normal, thus demonstrating that the tubules not only were not seriously injured, but also that they were able to care for the augmented output of nitrogen consequent upon the increased metabolism due to arsenic.

[19] Siegel, W., Ein Stoffwechselversuch bei Urannephritis am Hunde, *Zeit. f. exper. Path. u. Therap.*, 1907, iv, 561.

It is also noticeable that when vomiting occurred it followed immediately upon the administration of arsenic and is a manifestation of acute arsenic poisoning and not, as in uranium poisoning, an indication of renal insufficiency.

TABLE XVI.

Dog 12—Arsenic.

Date. 1909		Weight in gm.	Urine. Amount in c.c.	Urine. Spec. grav.	Urine. Total N in gm.	Urine. Total chlorides in gm.	Feces Total N in gm.	Feces Per cent. N.	Notes.
April	1	10540	510	1019	7.52	0.48	0.6363	4.64	No albumin.
	2	10600	530	1018	7.72	0.32	0.6363	4.64	
(1)	3	10640	550	1017	7.73	0.32	0.6363	4.64	0.03 gm. arsenic subcutaneously.
	4	10650	590	1019	8.30	0.24	0.6948	4.47	Vomited once; albumin +.
(2)	5	10660	560	1020	8.51	0.16	0.6948	4.47	0.02 gm. arsenic, subcutaneously; albumin +.
	6	10780	590	1018	8.43	0.16	0.7357	4.20	
	7	10800	450	1020	8.76	0.24	0.8934	5.82	Albumin +.
(3)	8	10780	600	1019	8.15	0.24	0.8934	5.82	0.03 gm. arsenic, subcutaneously; vomited.
	9	10590	600	1020	7.55	0.24	0.8934	5.82	
(4)	10	10600	480	1020	8.06	0.24	0.5895	3.43	0.03 gm. arsenic, subcutaneously.
	11	10620	500	1022	8.46	0.16	0.9560	3.57	Slight diarrhœa.
	12	10700	490	1020	7.73	0.12	0.9560	3.57	
(5)	13	10760	605	1015	7.72	0.26	0.8401	3.47	0.05 gm. arsenic, subcutaneously 9.30 a. m.; few hyaline casts. 4 p. m. severe vomiting; diarrhœa, collapse. Killed.

In the following experiment, in which uranium nitrate was administered at the height of the increase of nitrogen elimination due to arsenic, the effect of tubular injury on this elimination is strikingly shown.

The intravenous injection of arsenic caused for three days a marked increase of nitrogen, the daily average of which is, if the day following injection be omitted, equal to 37 per cent. of the normal average. On the last day of this period the simultaneous administration of arsenic and uranium nitrate is followed by a decrease of nitrogen which on the second day reaches the normal elimination. If we assume that the increased metabolism due to arsenic has continued, the decreased elimination of nitrogen can only be explained by insufficient elimination due to tubular injury.

TABLE XVII.

Dog 11—Arsenic—Uranium Nitrate.

Date. 1909		Weight in gm.	Urine. Amount[20] in c.c.	Spec. grav.	Total N in gm.	Total chlorides in gm.	Feces. Total N in gm.	Per cent. N.	Notes.
March	16	7820	495	1009	3.95	0.74	0.4464	2.93	
	17	7720	495	1013	4.08	0.72	0.4464	2.93	
(1)	18	7740	450	1010	3.67	0.68	0.4464	2.93	0.02 gm. arsenic in vein.
	19	7740	470	1016	3.53	0.52	0.4503	3.23	Slight vomiting; albumin +; no casts.
(2)	20	7640	305	1020	5.60	0.17	0.4137	2.94	0.02 gm. arsenic subcutaneously.
	21	7600	460	1012	5.51	0.16	0.3132	3.13	Albumin +; no casts.
(3)	22	7600	420	1012	5.01	0.24	0.3132	3.13	0.02 arsenic, 0.015 uranium nit. } subcutaneously.
	23	7480	495	1011	4.64	0.42	0.3331	3.41	Albumin +; casts +.
	24	7520	330	1019	3.85	0.32	0.3880	3.37	" "

Severe diarrhea developed on March 25 and the metabolism study was discontinued.

An attempt was also made to determine the nitrogen elimination in a second form of vascular nephritis, that due to cantharidin, but the excessive vomiting which follows the administration of this substance made accurate determination impossible.

We have then clear cut observations on uranium and arsenic nephritis, as types of tubular and vascular lesions, respectively, and results in chromate nephritis, approaching those of uranium, but not so clearly defined. Tubular nephritis as typified by uranium is characterized by a decreased elimination of nitrogen which is not a concomitant of the vascular type. This diminished excretion in tubular nephritis appears to bear a relation to the onset of gastro-intestinal disturbances, but as examination of the feces shows no increased elimination of nitrogen into the intestine, they cannot be due to nitrogenous toxic bodies vicariously eliminated as the result of the insufficient tubule function.

These observations are, therefore, of two-fold interest. In the first place, the work with arsenic offers additional evidence of the possibility of producing glomerular disturbance without affecting the function of the tubules; and, on the other hand, the diminished

[20] 500 c.c. of water daily by stomach tube.

excretion of nitrogen in tubular nephritis indicates the possibility of a retention not only leading to a disturbance of the glomerular activity in the "give and take" of kidney function, but perhaps also responsible for some of the more general manifestations of nephritis.

Salt Elimination.—The studies of the elimination of sodium chloride have not been entirely satisfactory. As shown by the tables the amount normally eliminated is small, and although there is some difference in elimination after the development of nephritis, as is clearly shown in Table XIII, the normal elimination of salt is, as a rule, so small that slight variations in absolute amounts assume relatively extraordinary proportions. The results are, therefore, given without comment. We anticipate that the results of a second series of experiments now in progress, in which the animals receive definite amounts of salt daily, will be more satisfactory.

SUMMARY.

By combining physiological and anatomical methods in the study of experimental nephritis it is possible to distinguish types of nephritis in which either tubular or vascular changes predominate, and are essentially characteristic of the lesion produced, but it is not possible to say that a given poison produces exclusively tubular or exclusively vascular lesions.

The so-called epithelial poisons (potassium chromate, uranium nitrate and corrosive sublimate) present anatomical evidence of extensive tubular injury, and in the early stages show, on physiological study, no evidence of vascular injury other than exaggerated contraction and dilatation of the vessels and increased diuresis. On the other hand, the vascular poisons, arsenic and cantharidin, which produce but little injury to the tubules, tend to cause anuria and are characterized by minimal contraction and dilatation of the vessels and little or no flow of urine. From the physiological point of view these two types may be, for practical purposes, considered as examples of tubular and vascular nephritis. They are not, however, pure types; for the increased diuresis of the early tubular type is in itself evidence of vessel irritability and similar to the increased irritability caused by small doses of vascular poisons, and

on the other hand, this essentially vascular lesion is accompanied by slight morphological changes in the tubular epithelium. Furthermore, the tubular lesions of chromium and uranium and corrosive sublimate pass into a stage closely resembling the vascular type, if not identical with it.

Two forms of late tubular nephritis may be recognized. One of these, the anuric form, is accompanied by severe gastro-intestinal disturbance and evidence of approaching anuria; physiological tests show diminished power of dilatation of the vessels and corresponding inhibition of diuresis. The second form, the polyuric, is characterized by a condition of polyuria up to the moment of anesthesia; physiological tests show that the power of dilatation is retained, but little or no diuresis occurs. Whether the vascular incompetency of the anuric form of late tubular lesions is a natural consequence of the vessel irritability seen in the early stages or is the result of the elimination of secondary poisons through the glomeruli cannot be absolutely determined. The peculiar impermeability of the glomerulus following anesthetization in the polyuric form, which is essentially a stage of recovery, we hope to explain by investigations now in progress.

Studies of the elimination of nitrogen show that in tubular nephritis, as represented by uranium nephritis, the output of nitrogen is considerably diminished. This apparently occurs also in chromate nephritis but is not clearly demonstrated. In both forms the onset of gastro-intestinal disturbances appears to bear a definite relation to the retention of nitrogen. On the other hand, as the nitrogen of the feces is not appreciably altered, these disturbances cannot be explained by the elimination through the intestine of toxic bodies of nitrogenous nature.

The nitrogen elimination in the urine in vascular nephritis as represented by arsenic nephritis is not only not diminished but is greatly increased as the result of the increased metabolism caused by arsenic. If at the height of this increased elimination, uranium nitrate is administered, the nitrogen output is markedly diminished. These observations demonstrate that not only are the tubular lesions in arsenic nephritis of little moment, but also that serious injury of the epithelium, as that due to uranium, may cause a temporary nitrogen retention.

A consideration of all the facts here presented allows us to conclude that although it is not possible to demonstrate that an experimental nephritis may be purely tubular or purely vascular, which is in accord with our clinical and pathological studies of human material, it is possible, if we combine the results of anatomical, physiological and chemical study, to recognize lesions which are predominantly tubular or vascular, or which change rather sharply from one to the other type, and are, therefore, of great value in the study of the problems of nephritis.

EXPLANATION OF PLATES.

[Owing to the length of the tracings, many of which are ten to fourteen feet long, only sections illustrating certain stages of the reactions are shown. In all tracings, the numerals, I, II, and III to the left, refer respectively to urine flow, kidney volume and blood pressure. The numeral IV is used to indicate the base line with signals. One inch of tracing corresponds to 18 seconds of time.]

PLATE XIV.

FIG. 1. This tracing illustrates the reactions caused by adrenalin, caffein, dog's urine and salt solution in the normal dog. (Compare with Tables I and II in text.)

Numerals at bottom correspond to sections of tracing: (1) showing the initial condition and the effect of adrenalin at 10:30; (2) the condition just previous to the injection of caffein at 10:41; (3) the increase of kidney volume and diuresis, fourteen minutes later, and also the effect of dog's urine; (4) represents the condition during a portion of the period from 11:01 to 11:21; (5) condition six minutes after the injection of five per cent. salt solution at 11:42; the diuresis in the meantime having fallen to six drops per five minutes. (Morphin and chloretone anesthesia.)

PLATE XV.

FIG. 2. This tracing illustrates the effect of caffein on the renal vessels in a state of increased excitability caused by a vascular poison (arsenic) given in dose insufficient to cause serious vascular injury. (Compare with Table VIII.) It serves also to illustrate the reactions of the first twenty-four hours after the administration of epithelial poisons (chromate, uranium and sublimate) which are in every respect the same. (Compare with Tables III, IV, V and VI.)

Numerals at bottom correspond to sections of tracing: (1) shows the initial condition at time of injecting caffein; (2) and (3) show the increase of kidney volume and diuresis eight and twenty-two minutes after caffein and in (3) the exaggerated reaction following the injection of dog's urine (morphin and chloretone anesthesia).

PLATE XVI.

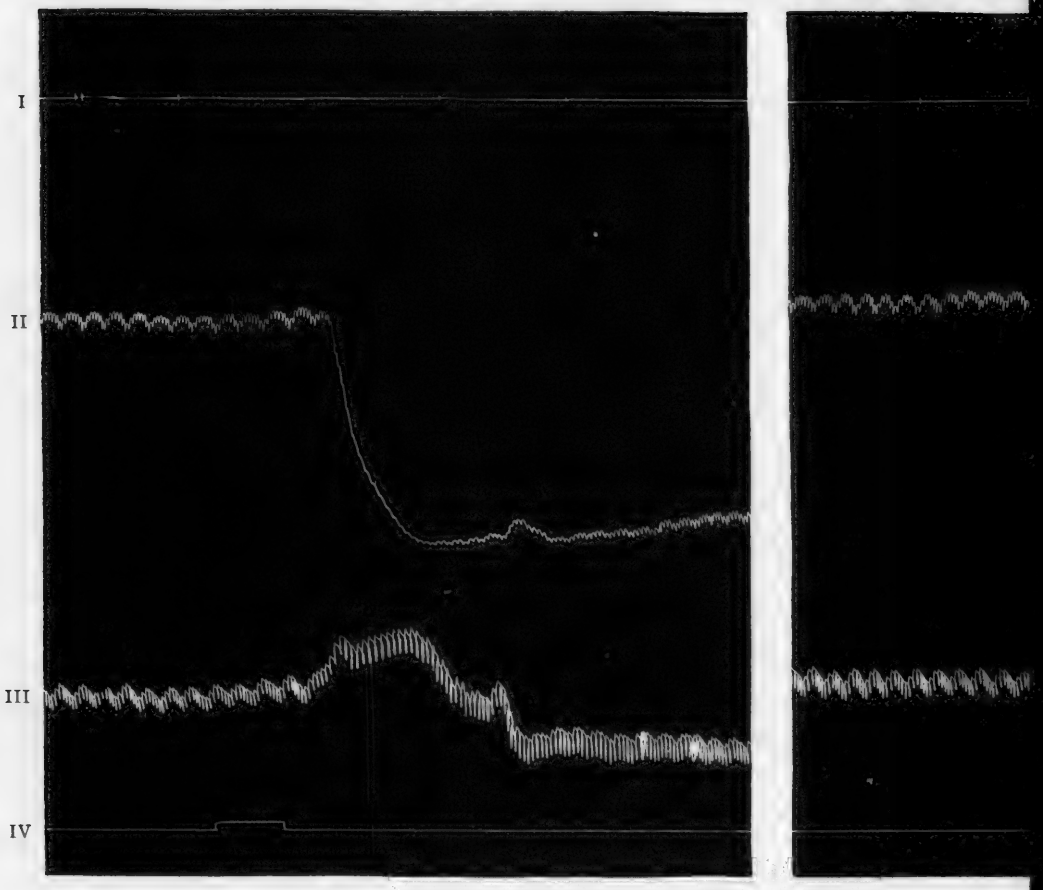
FIG. 3. This tracing illustrates the immediate effect of large doses of arsenic on the vascular reactions and on diuresis. (Compare with Table VII.)

Numerals at bottom correspond to sections of tracing: (1) shows the condition in normal dog just before the injection at 11:29 of 0.06 gm. of arsenic, subcutaneously (urine flow, four drops in five minutes); (2) reduction of kidney volume and suppression of urine as shown at 11:28; (3) condition at 11:39, ten minutes after the injection of caffein; (4) condition at 11:48, nine minutes after the injection of five per cent. salt solution; (5) effect of dog's urine at 12:04; (6) effect of adrenalin at 12:15; (7) end of experiment at 12:50, fourteen minutes after the additional injections of both caffein and five per cent. salt solution (ether anesthesia).

PLATE XVII.

FIG. 4. This tracing illustrates the condition in a late tubular nephritis on the fourth day of uranium poisoning; showing diminished power of dilatation of the renal vessels and diminished diuresis. (Compare with Tables XI and XII, which, however, represent more severe forms than that shown in tracing.)

Numerals at bottom correspond to sections of tracing; (1) shows initial condition and effect of adrenalin at 3:30; (2) effect of dog's urine at 3:45; (3) condition at height of caffein diuresis at 4:26, twenty-five minutes after caffein was injected; urine flow for five minutes (4:23 to 4:28) twenty-one drops as compared with a flow of eight drops in the twenty-five minutes previous to caffein injection; (4) condition at 4:47, thirteen minutes after injection of five per cent. salt solution; thirteen drops of urine per five minutes; (5) condition at end of experiment at 5:20; no diuresis (ether anesthesia).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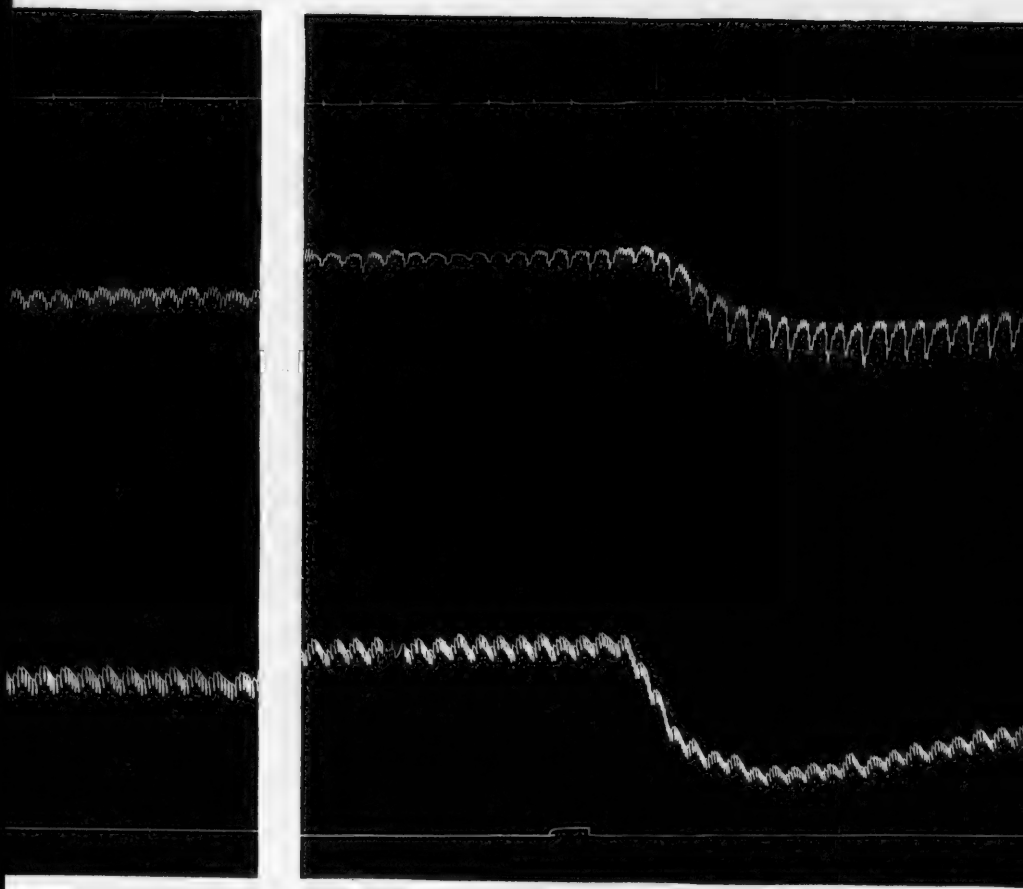

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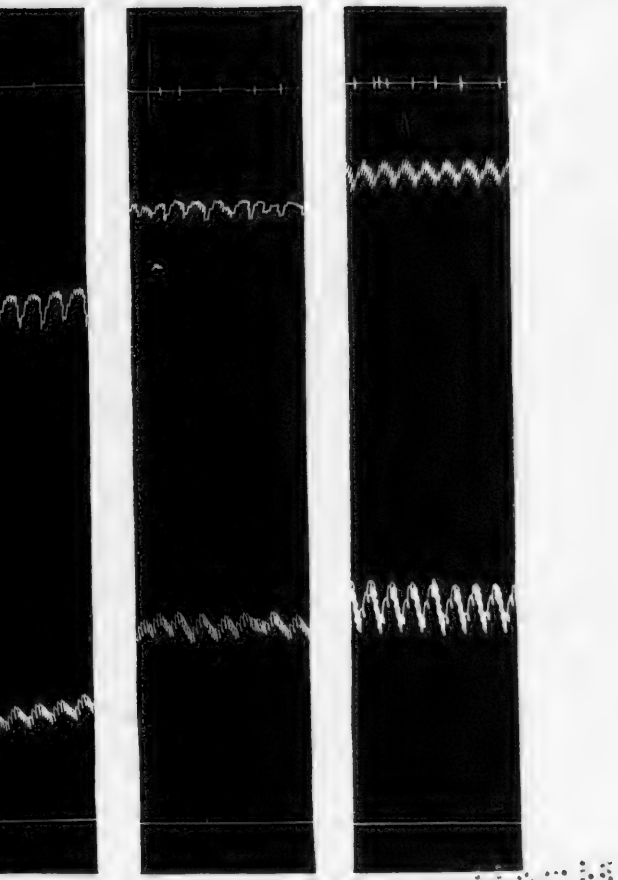
FIG. 1.

PLATE XIV.



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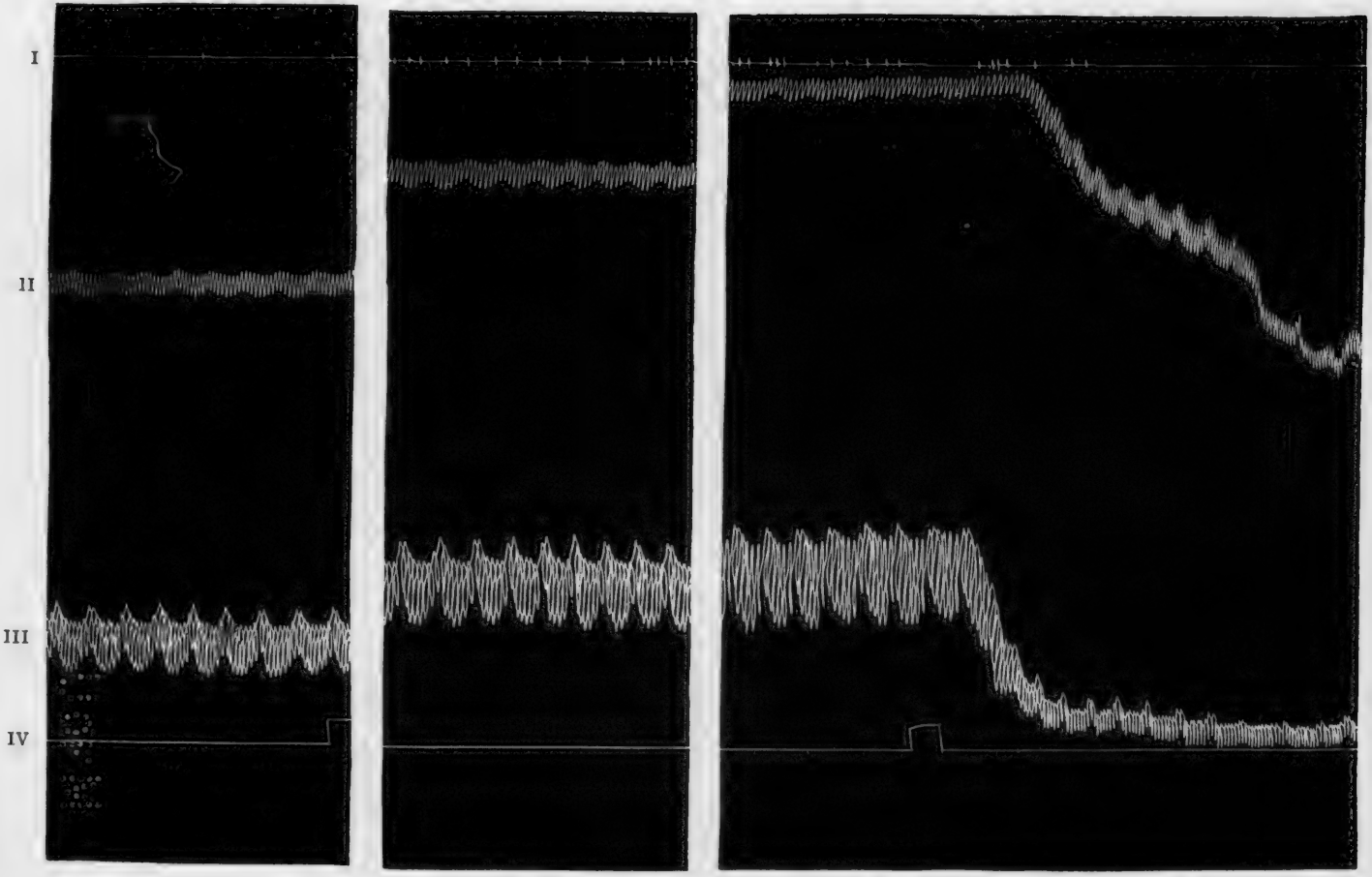
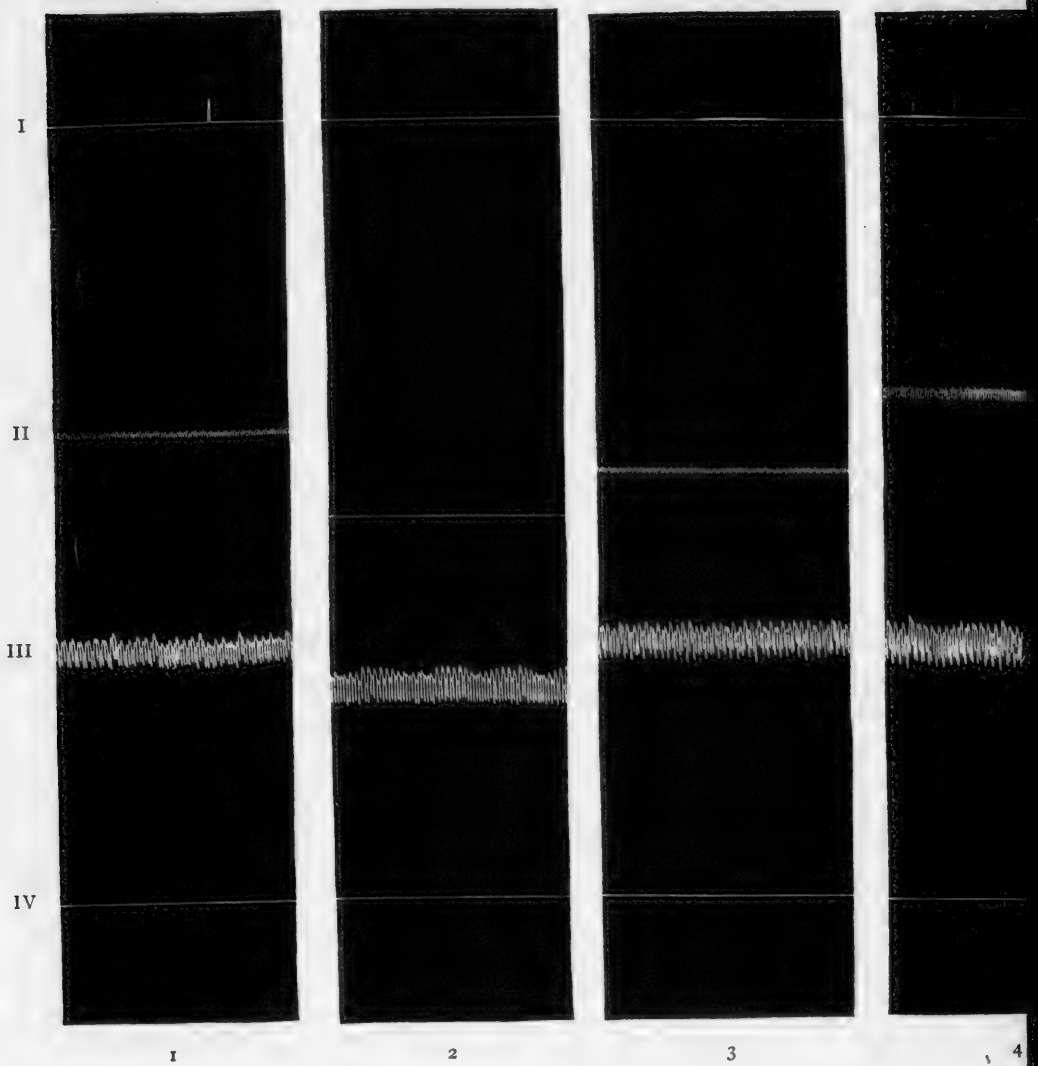


FIG. 2.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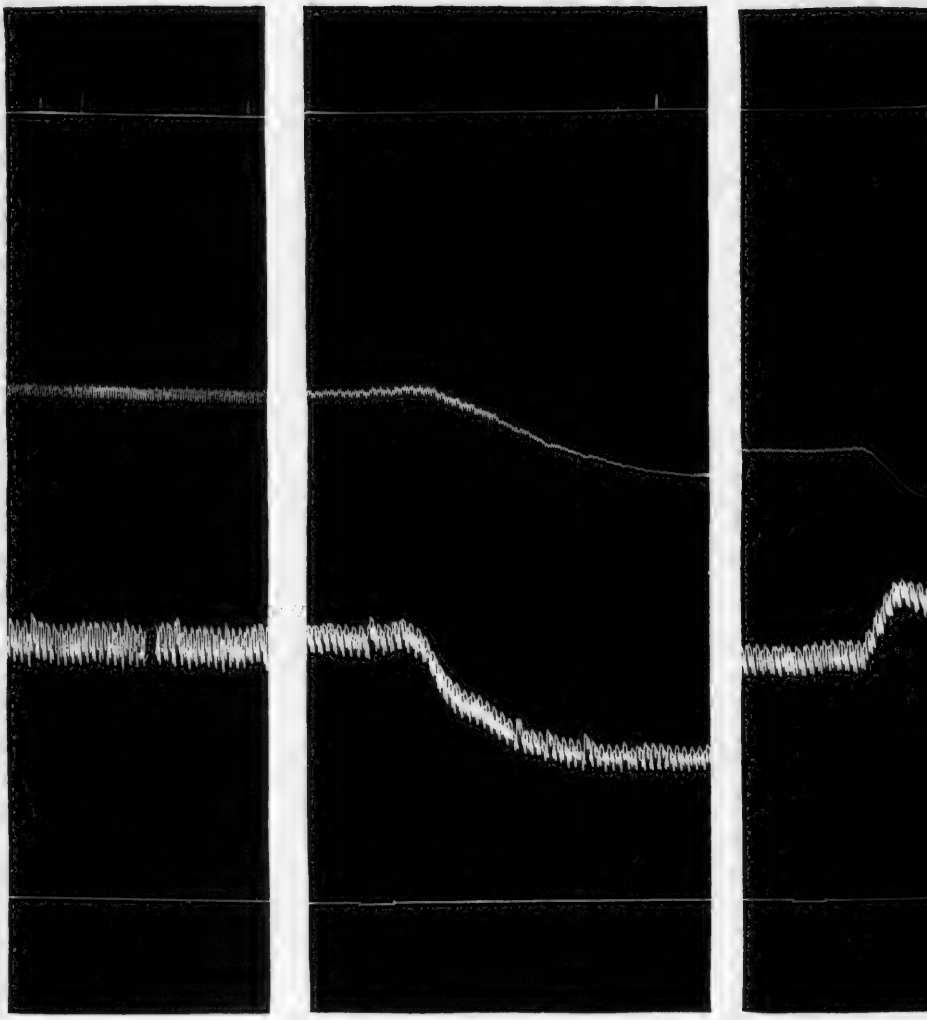
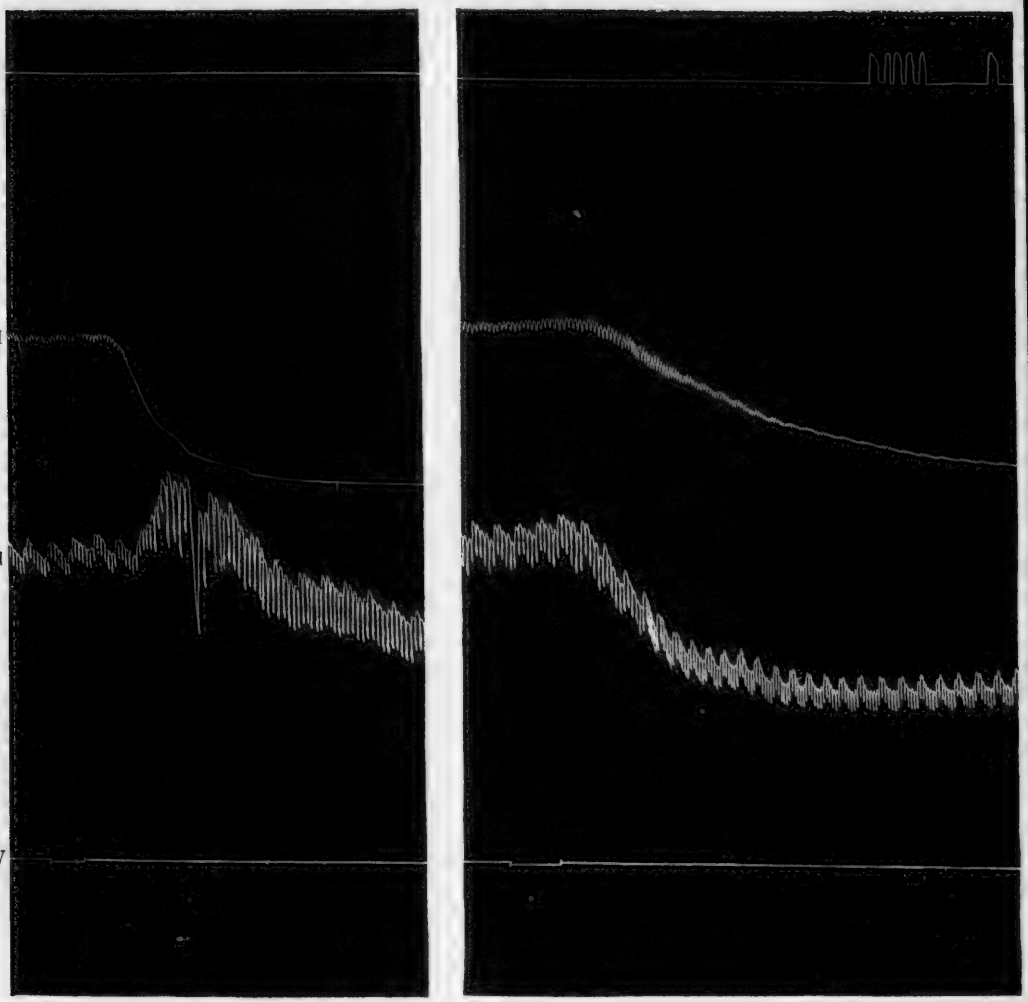


4 5 6

FIG. 3.

PLATE XVI.



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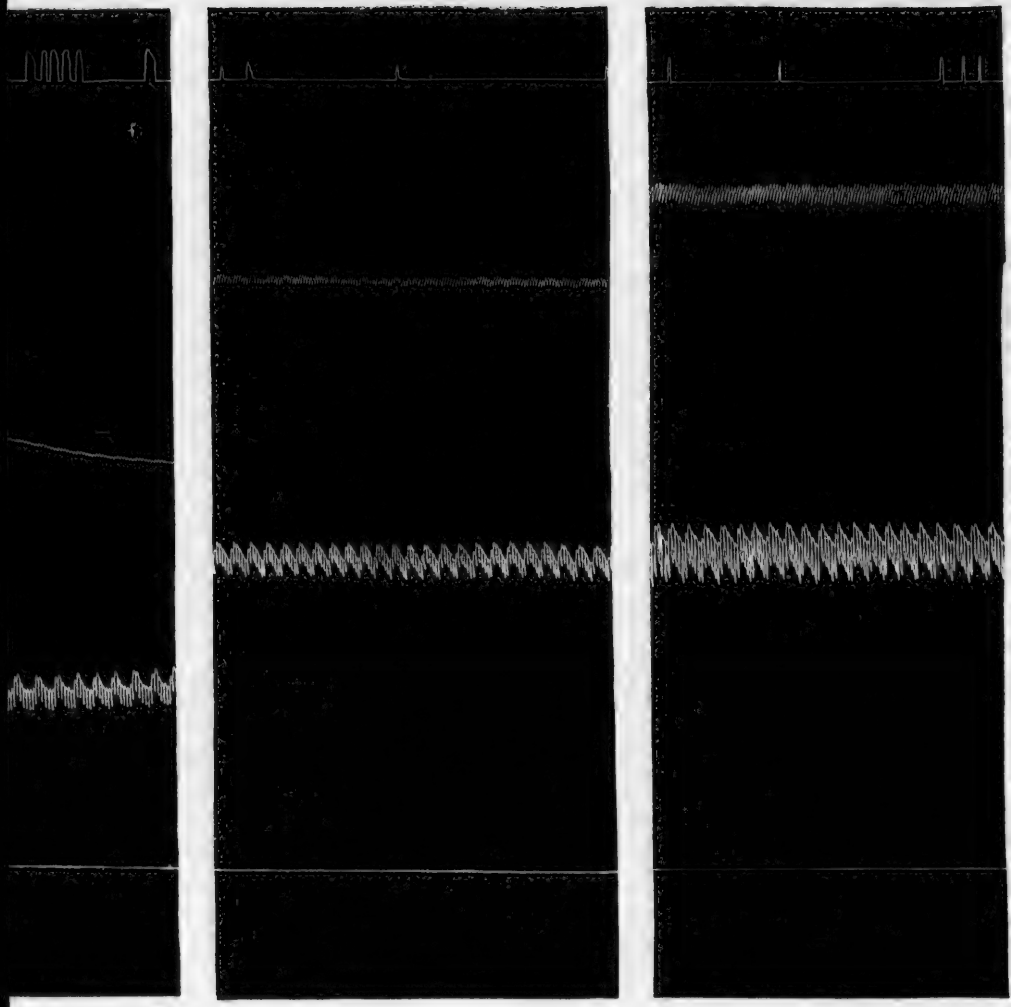
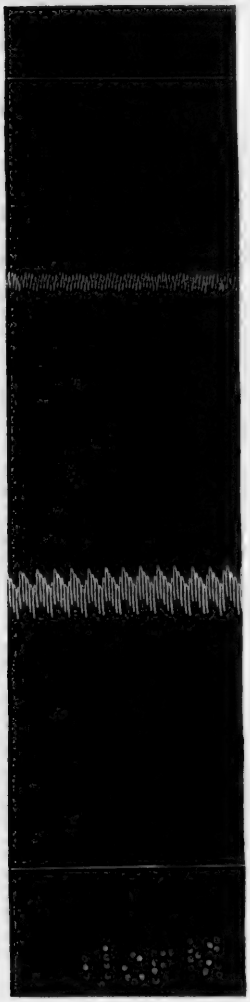


3 4

FIG. 4.

PLATE XVII.



5

EXPERIMENTAL EPIDEMIC POLIOMYELITIS IN MONKEYS.[1]

By SIMON FLEXNER and PAUL A. LEWIS.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

Plates XVIII and XIX.

INTRODUCTION.

Epidemic poliomyelitis has become, in the past decade, a world-wide disease. The present state of our knowledge of the epidemic spread of poliomyelitis, up to the outbreaks in Europe and America since 1907, is well given in Wickman's[2] monograph. That epidemic poliomyelitis is an infectious disease is clearly pointed out by Medin,[3] although, at an earlier date, Cordier[4] gave it as his belief that it is a contagious disease. The most convincing evidence of the contagiousness of epidemic poliomyelitis is supplied by Wickman's[5] studies of several Swedish epidemics.

Up to the present time there has existed no convincing knowledge of the nature of the agent causing epidemic poliomyelitis. Various bacteria and especially certain cocci[6] have from time to time been isolated in cultures from fluids obtained by lumbar puncture from patients suffering from epidemic poliomyelitis, or from specimens of the central nervous system removed at autopsy. These bacteria did not conform to one species or group of microörganisms and did not suffice to set up poliomyelitis in animals. They can be accounted for more satisfactorily as contaminations or secondarily invading bacteria than as the cause of the disease.

[1] Received for publication January 3, 1910.

[2] Wickman, Beiträge zur Kenntniss der Heine-Medinschen Krankheit, Berlin, 1907.

[3] Medin, Verhand. des x Internat. Med. Congresses, Berlin, 1890, ii, 37.

[4] Cordier, cited by Medin, *Lyon médical*, 1888, lvii, 5, 48.

[5] Wickman, *op. cit.*

[6] Geirsvold, *Norsk Magazin f. Laegevid*, 1905, iii, 1280 (cited by Harbitz and Scheel).

Since 1907, poliomyelitis has prevailed, during the summer months, as an epidemic in some parts of the United States. In 1907, the disease spread widely in the Eastern States;[7] in 1908, it appeared in the middle Western States, and in 1909, it attacked a large number of people in the Northwest (Minnesota, etc.) and reappeared in the East.[8] During this period, a similar epidemic appeared in Austria and Germany and by 1909 it had invaded France.

Our studies are limited to the two epidemics which affected New York and vicinity in 1907 and 1909. In 1907, one of us (Flexner) studied in various ways a number of fluids, obtained by lumbar puncture, from cases in different stages of the disease. The cellular nature, proteid strength, bacteriological content, and infectivity were investigated. The lymphocytes were increased in number, the proteid was not markedly increased, and aerobic and anaerobic cultivations were, as a rule, unsuccessful. Rarely, bacteria were present in the fluid; but the bacteria were not of uniform species. The fluids, obtained by lumbar puncture, were injected into laboratory animals, including monkeys, but without setting up any recognizable pathological condition. During the epidemic of 1907, we did not secure organs from a case of pure infantile paralysis and we failed, therefore, in our intention to inoculate monkeys from the spinal cord.[9]

In September, 1909, we secured the spinal cord from two cases of infantile paralysis in human beings. For these valuable specimens we are indebted to Dr. Ridner, of Lake Hopatcong, N. J., and Dr. Le Grand Kerr, of Brooklyn, N. Y. Dr. Ridner's patient died on the fifth or sixth day following the appearance of the paralysis, which affected the legs. The lumbar cord was obtained in a sterile condition, twenty-six hours after death, and employed for inoculation of animals. Dr. Kerr's patient had been widely paralyzed and died on the fourth day of the disease. The lesions in the cord were wide-spread and severe and affected gray matter and white. The entire spinal cord was obtained twelve hours after death and inoculated into animals four hours later.

[7] Committee Reports, *Jour. of Nerv. and Ment. Dis.*, 1909, xxxvi, 619.
[8] Kerr, *New York State Jour. of Med.*, 1909, ix, 484.
[9] Wollstein, *Jour. of Exper. Med.*, 1908, x, 476.

EXPERIMENTAL POLIOMYELITIS.

In order to favor the transmission of the disease to monkeys, we chose the brain as the site of inoculation, which was made under ether anesthesia, through a small trephine opening. After the operations, the animals were at once lively and normal. The injected material consisted, first, of emulsions in salt solution of the spinal cord from the children and, later, of emulsions and filtrates from the spinal cord and other organs from the monkeys developing paralysis.

Before proceeding to a detailed account of our experiments and results, it is desirable to review two publications, which immediately preceded ours, dealing with the experimental production of poliomyelitis. The first and more important is the report of Landsteiner and Popper,[10] which appeared in the summer of 1909, and which marks the beginning of the new era in the study of epidemic poliomyelitis.

The material employed for inoculation of two monkeys consisted of the emulsified spinal cord in salt solution obtained from a child nine years old, who died on the fourth day of attack from infantile paralysis. The emulsion was injected into the peritoneal cavity of the monkeys. One of the latter became severely sick on the sixth day and died on the eighth day after inoculation. The other monkey became paralyzed on the seventeenth day and was killed on the nineteenth day after inoculation. The spinal cord of the first monkey was not used for further inoculation, while that from the second monkey was used to inoculate, probably by intraperitoneal injection, two other monkeys that did not, however, develop the symptoms of the disease. Apparently they were unaffected by the injection.

The anatomical examination of the first monkey showed normal internal organs, except the spinal cord which presented severe lesions. The microscopical examination of the spinal cord gave the following result: the pia was infiltrated with small, round, deeply-staining cells and the infiltration extended chiefly along the median anterior fissure, and less along the median posterior fissure into the cord and medulla, and into the brain. The substance of the cord showed throughout areas of inflammation, which were most marked at the cervical enlargement. These areas appeared either in the form of heavy perivascular infiltrations or as more diffuse infiltrations in the substance of the cord. An edema was also present. The diffuse infiltrations occurred chiefly in the anterior gray matter, and to a less extent in the posterior gray matter. Hemorrhages were also present in the gray matter. Similar lesions, but of less intensity, were found in the region of the medulla, pons and brain stem. The ganglion cells of the anterior horns especially showed severe degeneration and were often surrounded by, or actually invaded with, mononuclear and polynuclear cells.

[10] Landsteiner and Popper, *Zeit. f. Immunitätsforsch., Orig.*, 1909, ii, 377.

The anatomical findings of the second monkey were similar to, but less intense than, those in the first. The lesions were most pronounced in the lumbar region, while the thoracic and cervical portions were little affected. The microscopical appearances were similar to those of the first monkey. Hemorrhages were, however, absent and the infiltration of the substance of the cord showed fewer polynuclear cells.

Owing to their inability to transmit the disease from one monkey to another, Landsteiner and Popper discuss the three following possible explanations of the failure, namely: (1) whether the disease in the first monkey was caused by a transferred poison or by the infectious agent; (2) whether a successful transfer from monkey to monkey might not have been secured had the cord of the monkey, dying on the sixth day of the disease, been used; and (3) whether the infectious agent may not have become attenuated in virulence in its passage through the monkey and thus have lost its power of further transmission. They inclined to the last explanation.

At the time our studies of 1909 were begun, we were familiar with the foregoing publication; but before our first report[11] appeared in print, a second account of a successful transfer of poliomyelitis to a monkey was published from Vienna, whence the first appeared, by Knoepfelmacher.[12]

On December 22, 1908, 14 hours post-mortem, the spinal cord was removed from a child of one year, who had died on the sixth day of the disease, and 10 centimeters of the cervical portion, after emulsification in salt solution, were injected into the peritoneal cavity of a *Macacus rhesus*. The first evidence of sickness was noted on the eighth day, and the first evidence of paralysis on the twelfth day, after the inoculation. On the fourteenth day the animal was killed with chloroform and 10 centimeters of the spinal cord were rubbed up in salt solution and the emulsion was injected into a second monkey (presumably by way of the peritoneum) which was unaffected. The anatomical and histological findings agreed with those described by Landsteiner and Popper.

CLINICAL FEATURES.

We have observed with care the monkeys inoculated successfully with the virus of epidemic poliomyelitis and have classified them into groups according to the symptoms recognized. The descrip-

[11] Flexner and Lewis, *Jour. of the American Med. Assn.*, 1909, liii, 1639.

[12] Knoepfelmacher, *Med. Klin.*, 1909, v, 1671. Strauss and Huntoon (*New York Med. Jour.*, 1910, xci, 64) have since reported a successful intraperitoneal inoculation of a monkey and failure to transmit the disease further.

tion to be given is based upon a study of 81 monkeys which became infected with the virus.

Incubation Period.—The incubation period has been taken to be the interval elapsing between the time of inoculation and the appearance of the first definite paralysis. During this interval certain other clinical manifestations of illness may be noted and these will be considered separately as prodromal symptoms. The latter besides being inconstant call frequently for the exercise of personal judgment for their detection and hence they can be defined less accurately than the paralysis. In several instances the monkeys fell ill and died without paralysis having occurred or been noted. These animals were included among the series of successful inoculation only when the typical lesions were present in the nervous system. The incubation period in them was calculated from the inoculation to the onset of definite symptoms of illness.

The shortest period thus far noted as elapsing between the inoculation and the onset of paralysis has been 4 days and the longest period, 33 days. The average period has been 9.82 days. The number of animals developing paralysis after the twelfth day was 16, and the number developing paralysis before the eighth day was 18. Hence the extremes of the incubation period are probably simple variations from a mean which represents the activity of the virus for monkeys. We possess some evidence that the virus may suffer, in certain strains, an enduring alteration through which its activity is diminished. When the site chosen for inoculation is some other than the brain, or the material used some other than an emulsion of the spinal cord, the incubation period has not usually been at either extreme period.

Prodromal Signs.—The inoculation of the virus into the brain or other parts produces no immediate effects. As soon as the effects of the anesthetic disappear, the monkeys appear normal. This condition persists until a period of from six to forty-eight hours before the onset of paralysis when certain abnormal signs may be noted. The animals become nervous and excitable; on being disturbed and made to move about the cages, they tire quickly; a tremor of the head, face or limbs develops; when the attention can be attracted the gaze is shifting, rather than fixed as in the normal monkey, and

the face is wrinkled and mobile rather than smooth and placid; the hairs are erected somewhat, and the animals prefer to remain quiet. All these symptoms are almost never noted in a given animal and they occur in varying combinations. The temperature does not rise constantly during the incubation period and gastro-intestinal symptoms rarely occur. A few animals have shown diarrhœa but this condition may well have been a coincidence.

Onset of Paralysis.—Either with or without the premonitory signs, the paralysis develops suddenly. In general it may be stated that any of the larger groups of voluntary muscles may be first involved. The table shows the frequency with which the chief types of paralysis developed in our series at the onset.

TABLE I.

Frequency of Chief Paralyses at Onset.

Location of Paralysis.	No. of Animals Affected.	Total.
Both legs	20	
Right leg	8	
Left leg	12	40
Both arms	3	
Right arm[13]	9	
Left arm	9	21
Four limbs	10	10
Right leg and left arm	1	1
Neck[14]	1	1
Bulbar or cerebral[15]	8	8
Total	81	81

Concomitantly with the appearance of paralysis of any large muscle group, other muscle groups are found either weak or partially paralyzed. The exceptions to this rule are present among the cases of slight affection. The onset of symptoms in the bulbar or cerebral group has not been the same in any two cases,[16] and death

[13] One animal showing paralysis of right arm was chiefly a case of bulbar affection.

[14] In six instances the back or neck was involved as soon as any limb.

[15] The eye muscles were paralyzed once in a case of cerebral affection. Facial paralysis has occasionally been seen but never at the first manifestation up to the present time.

[16] Illustrative protocols are given at the end of the article.

has occurred before the developments of flaccid paralysis. The striking features of the cases have been spasticity of the limbs, marked incoördination resulting in violent pseudo-convulsions; epileptiform convulsions with tonic and clonic muscle-spasms; and, finally, sudden death of apoplectiform type. Death has occurred within thirty minutes of the first appearance of cerebral symptoms.

Usual Type of Paralysis.—The rule is for the paralysis to extend quite rapidly to other muscle groups. In mild examples of the affection, the extension may be slight or pass unnoticed. In examples of moderate severity, the extension soon ceases. In the usual examples, which are severer than these, the paralysis extends, with greater or less celerity, from one limb to another, to the back and the neck, until death supervenes. The paralysis, when developed, is of the flaccid variety. The severely affected animal lies recumbent, the respiration being feeble and shallow and the temperature subnormal. When the condition is one of moderate severity, the progress of paralysis has reached its limit in about twenty-four hours, after which it remains stationary or recedes, or death occurs. In the severer conditions, all the muscles, possibly excepting those of the neck, are completely paralyzed in twenty-four, or at most forty-eight hours. The progress of the paralysis is usually, if not always, outlined in advance, by weakness of the muscles next to be affected. There is no rule observed of ascent or descent, but the extension occurs in both directions. The period elapsing between the onset of paralysis and death ranges from one to six days.

In several instances, the weakness of certain muscle groups, accompanying the paralysis of others, has been replaced by a spastic state producing contractions of the limbs or retraction of the head. This condition usually has not been intense, since the muscles would be relaxed when the animal attempted to use them.

The investigation of the sensory disturbances has been far less satisfactory. Both what appeared to be areas of anesthesia in some animals, and hyperesthesia in others, were noted. The paralyzed limbs are usually colder than the non-paralyzed, and the circulation is sluggish in them. One monkey, which became affected, gave evidence of pain in the feet for forty-eight hours preceding the onset of the paralysis. When the paralysis was developed, the pain had disappeared.

The mental condition of the animals remains unaffected even in the severe forms of the disease. The temperature, in severe cases, is definitely subnormal.

Termination.—This division of our subject cannot be discussed with the completeness of the other sections. Since the main object of our study required that we maintain an active virus, we have been obliged to sacrifice a considerable number of the affected animals within twenty-four to forty-eight hours of the onset of the paralysis.

The affected monkeys may recover. When this happens, the paralysis reaches a maximum, becomes stationary, and then recedes more or less. When the affection has been severe, the animals appear sick for several days, but when recovery commences the general symptoms of sickness quickly disappear. The muscles which were weak, but not definitely paralyzed, regain strength. Hence, the actual paralyses are more sharply demarcated. Although the variations are considerable, it happens that within a week of a severe or critical state the animal has regained health and general strength, except for the actually paralyzed muscles. In other instances, two or three weeks have not sufficed for the restoration of strength to muscles apparently intact. In some instances in which the paralysis affecting a single limb appeared to be complete, it has entirely disappeared within a few weeks.

When paralysis has been marked it usually persists. There has not yet been sufficient time to show what the end result of these cases will be; but present indications, based on several animals paralyzed from three to ten weeks, are that further recovery will be very slow and incomplete. Within a month or so, the paralyzed limb tends to stiffen and to contract, the kind of contracture depending somewhat upon the position assumed by the paralyzed limb in the meantime.

The animals may die. When the first force of the affection is exerted upon the medulla, death may occur within a short time of the first appearance of symptoms. When the limbs are first affected, the progress and extension of the paralysis may be very rapid and death be caused quickly through involvement of the medulla. Again, the progress may be slow and the prostrated

animal may gradually grow weak and die after one or several days. When the period of disease is prolonged, then other factors, such as secondary infections and general nutritive and trophic disturbances, must be considered.

The accompanying table exhibits the mortality in the cases considered in this paper. It requires a few words of explanation, since, while a part of the recoveries and deaths noted are facts, another part are surmises. Each set is clearly indicated. The necessity for this division has been explained already and was caused by the need of maintaining an active virus for inoculating other animals. We are of the opinion that we have put the number of probable recoveries rather too high, but this was done to avoid over-statement of the probable fatality of experimental poliomyelitis in monkeys. When the probable outcome of certain sacrificed animals could not be predicted, the animals were excluded from the tabulation.

TABLE II.

Showing Probable Mortality of Experimental Poliomyelitis.

Termination of Disease.	No. of Animals.	Total.
Actual death	23	
Probable death	21	44
Actual recovery	9	
Probable recovery	7	16
Uncertain	21	21
Total	81	81

From the figures given it follows that in 54.3 per cent. of the monkeys, in this series, which developed poliomyelitis, the issue would in all probability have been fatal. Hence, the experimental disease is more highly fatal than is the spontaneous disease in human beings.

PATHOLOGICAL ANATOMY.

The spinal cord and its membranes show, in fatal cases, visible lesions of varying intensity. The pia mater is usually congested, the hyperemia being most apparent at the lumbar and cervical enlargements. An edema is not perceptible and the cerebrospinal fluid is not appreciably increased. The consistence of the cord is rarely altered except in autopsies made some hours after death, when it is

softer than normal. The latter condition usually is of post-mortem development and autolytic in nature. Definite lesions of the white matter were not made out, while it is common to find the gray matter altered. The chief lesions consist of an edema (excessive moisture of surface), diffuse vivid injection of the blood-vessels and punctiform or larger (pin-head) hemorrhages. The hemorrhages are disclosed less well by transverse than by median incision. In the latter preparations, in which the gray matter is exposed, the hemorrhages are seen to follow the course of small vessels. The lesions are not diffuse or uniform but they tend to be most marked in those regions of the spinal cord corresponding with the paralyzed groups of muscles. And yet, it would be wrong to suppose that the lesions were strictly limited to these regions, for, as a matter of fact, they are much more widely distributed.

The macroscopic lesions are far less regularly present in the monkeys killed soon after paralysis has set in. In some of this class of animals, no lesions were visible; but the greater number show lesions not unlike those present in the animals dying spontaneously.

The lesions of the medulla, when present, resemble those of the spinal cord. The congestion is, however, more apparent than the hemorrhages, although the latter, of small size, have been seen.

Gross lesions of the brain have been observed rarely. In a few instances, hemorrhages along the needle-track have been present, but they were of traumatic origin and are not to be regarded as part of the pathological anatomy of the experimental disease. In one or two instances, small cysts with clear contents were found at the inoculation-site long after the operation. They gave rise to no symptoms. The substance of the brain has been excessively moist in some instances; and in a few cases yellowish dots, not clearly distinguishable, have been noted which, on microscopic study, proved to be areas of necrosis. There occurred, a few times, small subdural hemorrhages, present on one or both sides.

The appearance of the cord is somewhat different at a later stage, when healing has set in. Then the lesions, when easily visible, are firmer, paler, more sharply circumscribed, and raised somewhat above the surrounding gray and white matter. No

instance has yet been examined in which the lesion was more than a couple of weeks old, so that the ultimate appearance of the scar cannot be described.

To resume, therefore, the lesions in the spinal cord and medulla of the monkeys, visible to the naked eye, consist of congestion and hemorrhage into the gray matter chiefly, but not exclusively confined to the anterior horns. The general appearances of the spinal cord, medulla and brain are not greatly altered, and the visible effects are no proper measure of the damage inflicted by the virus.

PATHOLOGICAL HISTOLOGY.

The general descriptions of the lesions revealed by the microscope will be discussed in respect to the spinal cord and brain and as they relate to the membranes, white and gray matter, and to the intervertebral ganglia.

Spinal Cord.—The lesions are more severe and widespread in the spinal cord than in the brain. In point of severity the lesions are most pronounced in the gray matter and membranes and least marked in the white matter. As a rule, no part of the spinal cord, including the medulla, is entirely free from lesions; but in respect to severity there is considerable difference at various levels. As could be predicted, the severest lesions tend to occur at the levels corresponding to the most marked paralysis of muscle groups; but there is room for discrepancy here since the paralysis of certain muscle-groups (leg or arm) is more apparent than of others (back or neck). In this way, we would account for the severe lesions, sometimes present in the thoracic cord although the effects of the lesions were not very apparent.

The meninges usually showed a more or less diffuse infiltration with round cells. The layer immediately next to the white matter of the cord tended to show more cells than the layer next to the dura mater. The greatest accumulations of the cells were about the blood-vessels (arteries and veins), the sheaths of which were surrounded by a thick collar of the cells. The muscular coats and the intima remained intact, although the lumina were often encroached upon by reason of compression of the vessels. When the vessels were small the effects on the lumina and hence upon the permeability were considerable.

The infiltration of the adventitial and perivascular lymph sheaths and adjacent pial membrane continued as the membranes and contained vessels entered the fissures and extended into the substance of the cord; and often the degree of infiltration was greater in the intramedullary parts of the membranes and vessels (Plate XVIII, Fig. 1). The height of the infiltration, now of the lymph sheath, was reached in the gray cornua.

In the early stages of the paralysis all the infiltrating cells were round, showed dark, nearly solid nuclei and scant protoplasm, and resembled lymphocytes. Later on, when the reparative process had set in, the lymphoid type of cell was replaced by spindle cells, possessing processes resembling fibroblasts. The affected vessels, moreover, now showed still greater diminution of caliber and excess of spindle nuclei in the muscular coat.

Nothing remained in the membranes to indicate either the quality or the quantity of the cerebrospinal fluid; but specimens removed by lumbar puncture at different periods taught that at the onset of paralysis the fluid was sometimes under slightly increased pressure and sometimes under very low pressure, was faintly opalescent, contained an excess of proteid and was spontaneously coagulable, and further contained a large excess of lymphocytes. This condition is of brief duration and is succeeded by one in which the fluid is clear and non-coagulable, the proteid not increased,[17] only the lymphocytes being more numerous than normally. In course of the healing process the lymphoid cells of the pia-arachnoid are also substituted by spindle cells.

The meningeal cellular invasion is always interstitial and does not give rise to an exudate upon the surface of the cord or brain such as occurs in acute exudative inflammations. There is, indeed, a notable absence of polymorphonuclear leucocytes. On the other hand, the cellular infiltration does extend along the extramedullary nerve roots, affecting their pial investment and penetrating into the connective tissue between the nerve fibers. The impression is gained that the posterior roots are infiltrated to a greater degree and more frequently than the anterior roots. The change from round to

[17] This determination is made with Noguchi's butyric acid test. *Jour. of Exper. Med.*, 1909, xi, 604.

spindle cells, in course of reparation, also takes place in the nerve roots.

The white matter of the cord is not spared, but in point of frequency and severity of affection it occupies an inferior position. The lesions observed were the following: edema; perivascular cellular infiltration which was sometimes of a high degree and penetrated beyond the lymph sheath into the nervous tissue; hemorrhages; degeneration or necrosis of nervous tissue, associated with cellular invasion and fragmentation of nuclei or hemorrhage; and focal accumulations of lymphoid cells, independent of vessels or necrosis. The lesions in the white matter were always focal and occurred indifferently in the various columns. Often no connection was discovered with lesions of the gray matter and sometimes a continuous cellular infiltration, with or without degeneration, extended from gray into white matter. The lesions tended to occur near by rather than at a distance from the gray cornua.

The gray matter was invariably affected. The lesions involved the anterior and posterior horns and the commissure, and they affected the blood and lymphatic vessels, the ground substance and nerve cells.

The anterior horns were, as a rule, more severely and widely injured than the posterior horns. The vessels and arteries, especially in the anterior gray matter, showed a high degree of cellular infiltration of the perivascular spaces together with edema of the spaces, which often spread them widely apart and destroyed the fibrillar meshwork, and hemorrhage into the spaces. From the vessels, the cells often passed into the ground substance. But independent foci of small cells, edema or hemorrhage also existed in the nervous tissue. The nerve cells often showed degenerations which consisted of hyaline transformation and necrosis, leading to loss of tigroid substance, cell-processes, nuclei, etc. Sometimes merely a contracted nucleolus remained stainable. Often the cell was surrounded by lymphocytes or invaded with polynuclear leucocytes. It happened that the nerve cells had disappeared and leucocytes occupied their places. Not all the nerve cells, in a segment, would show equal degeneration, and in rare instances only was the entire width of the anterior horn degenerated. Ultimately, a

part or all the nervous elements would be removed and replaced by an indefinite cellular tissue, containing many compound granular corpuscles (Plate XIX, Fig. 3).

The commissures, Clarke's columns and posterior horns and the blood vessels entering them, showed similar if less profound lesions.

The infiltration of the perivascular sheaths of the vessels was continuous with that of the pia-arachnoid and especially the pial extensions into the spinal cord. The number of polymorphonuclear leucocytes in different specimens varied considerably, but they rarely if ever were as numerous as the lymphocytic cells.

The lesions in the spinal cord were more abundant and of severest grade in the lumbar and cervical enlargements.

The medulla oblongata exhibited lesions similar to those of the cord and affecting the membranes and substance. The blood vessels, nuclei and ground matter were all involved. The foci were, as a rule, smaller than those in the spinal cord.

The brain showed lesions which were, however, more sparsely scattered than in the cord. The meninges showed infiltrations extending between the sulci and along the vascular sheaths into the brain tissue. Focal necrosis of cells, lymphocytic emigration, and hemorrhages occurred in the cortex and white matter, in the basal ganglia and in Ammon's horn. The ventricles showed no lesions.

When the virus was confined mechanically to a given part of the brain, the vascular infiltration was intensified. This occurred in connection with the inoculation of the glycerinated virus into the brain substance. When degeneration and necrosis of cells were produced by the injection of the virus into the brain (as rarely happened) the perivascular infiltration was intensified, possibly because of a freer development of the virus in the necrotic tissue.

The blood-vessels and arteries, especially, *in the subcutaneous tissue,* at the site of the nodule caused by a subcutaneous inoculation, showed infiltration with round and spindle cells similar to those present in partly healed lesions in the spinal cord. Other parts of the subcutis were quite free from infiltration.

The intervertebral ganglia are regularly involved in the process. The involvement consists of a diffuse and nodular infiltration with

lymphocytic cells which collect between the nerve cells and about the nerve fibers, and degeneration and necrosis of single ganglion cells. These cells either become completely necrotic and hyaline and appear pink and homogeneous and devoid of nuclei, or they show contracted nuclei and altered eosin staining with loss of tigroid markings. The degenerated cells become invaded with or replaced by leucocytes and lymphocytes (Plate XVIII, Fig. 2).

AVENUES AND SOURCES OF INFECTION.

The intracerebral mode of infection is not the only successful one. It has been determined that the virus of epidemic poliomyelitis when introduced into the body by means of the blood, subcutis, peritoneum, spinal canal and large nerves, tends to localize in the spinal cord and brain and set up the specific lesions, in the same manner as when injected into the brain. It remains to be determined whether these several routes give uniformly as good results as the intracerebral route. Our impression is that infection is readily accomplished by way of the subcutis and less readily by way of the peritoneal cavity. We have made several unsuccessful attempts to produce infection through intratracheal inoculation and by feeding. But the number of experiments will have to be considerably greater before a final conclusion can be ventured on these points. On the other hand, it has been shown that the cerebrospinal fluid, at the beginning of the paralysis, is capable when injected into the brain, of setting up paralysis in other animals. Hence at this period, at least, this fluid contains the virus.

The blood contains the virus at the beginning of the infection, but how richly has not been accurately determined. As little as two cubic centimeters may fail to cause infection while as much as twenty has caused typical paralysis. After a successful subcutaneous inoculation with an emulsion of the spinal cord, the regional lymphatic glands (axillary and inguinal) and the subcutaneous nodule resulting proved infectious; and the paralysis developed earlier from the gland than the nodule.

The naso-pharyngeal muscosa also contains the virus. The excised membrane, including the tonsils and turbinate mucosa, on

being rubbed up with quartz sand, macerated and filtered, yielded a fluid which, on being injected into the brain, caused paralysis. Thus far infection has not been secured with the spleen, bone-marrow, liver or retro-vertebral glands.

NATURE AND RESISTANCE OF THE VIRUS.

Film preparations and sections prepared from the spinal cord of human beings and monkeys examined, prepared and stained in the most various ways, showed no parasites, either bacterial or protozoal, that could account for the infection. The readiness with which epidemic poliomyelitis could be transmitted to monkeys and the absence of visible and stainable parasites, early suggested the possibility that the infectious agent belonged to the minute filterable viruses. To determine this point, the spinal cords, removed from monkeys just paralyzed, were triturated with sterile quartz sand, thoroughly shaken, and pressed through a Berkefeld filter which had previously been tested and found bacteria-tight. These clear and bacteriologically clear filtrates have been used repeatedly to inoculate monkeys, both by the intracerebral and subcutaneous routes, and have regularly caused paralysis. From these paralyzed animals, the virus has been transferred to other monkeys, so that it can be asserted that the effects it produces are caused not by a filterable toxic substance but by a true virus or living microörganism.

It was because of the fact of the filterability of the virus that we succeeded in detecting the presence of the organism in the muscosa of the mouth and nose.

The clear fluids obtained by filtration when examined under the dark field microscope show innumerable bright, dancing points devoid of definite size and form and not truly motile. This fluid prepared and stained by means of Loeffler's flagella stain shows minute particles of roundish or oval form which were absent from a similar filtrate prepared with the nervous system of a rabbit. That the particles represent the microörganism of poliomyelitis cannot be affirmed at present.

The filterable viruses of other diseases are resistant to injurious agencies. In common with some of them, we have ascertained

that the virus of poliomyelitis, as contained in the comminuted spinal cord, withstands glycerination for at least seven days and resists drying over caustic potash in a desiccator for the same period.

Moreover, it has been found that the virus retains its virulence apparently unimpaired on being kept constantly frozen at — 2° to — 4° C. in the Frigo apparatus for a period of at least forty days, and also when kept for at least fifty days at a temperature of about + 4° C., in course of which time the latter specimen of spinal cord became slowly softened through autolysis and overgrown superficially with mould.

The virus appears to be readily injured by heating. Using the filtrate to avoid the effects of coagulation of the proteid, we have found that a temperature of 45° to 50° C., maintained for half an hour, sufficed to render the filtrate incapable of causing paralysis when injected into the brain.

The parasite of epidemic poliomyelitis is, therefore, very minute and cannot, for the moment, be further classified, since the precise position among living things held by the filterable viruses has not been determined. At least one of these viruses, that of pleuropneumonia of cattle, has been cultivated artificially. An attempt has been made to cultivate the virus of poliomyelitis in test tubes.

The result has seemed to be successful, but it is still in doubt. What has thus far been determined is that the filtrates when added to bouillon prepared with human blood serum or ascitic fluid become clouded and retain their virulence for four days in the thermostat. The transfer of a fraction of a cubic centimeter of such turbid fluids to a second set of tubes may cause a turbidity to develop in them. The staining of these turbid fluids with Loeffler's flagella stain brings out the oval or roundish particles in numbers. But thus far we have not induced paralysis by inoculating the culture fluid of the second or third transfer of the original virus.

IMMUNITY.

Experiments have been and are still being conducted to determine the kinds and degrees of immunity which are produced by the inoculations of the virus. Since the literature on epidemic

poliomyelitis is silent on this subject it can be inferred that a second attack of the disease is rarely if ever suffered by one individual. Two possible reasons can be assigned for this: the first and most probable is that one attack of this disease, as with other acute general infections, tends to afford an enduring immunity; and the other, that as epidemics occur infrequently and reappear after long intervals, the children once affected have passed beyond the susceptible age period at the time of the next epidemic.

We have attempted, and up to the present unsuccessfully, to reinfect monkeys which had recovered or were recovering from the disease. Thus far ten monkeys have been subjected to reinoculation, into the brain, at periods varying from eight days to two months after the first paralysis appeared. The larger number of reinoculations were made at the end of the first month following the paralysis.

In point of severity the first attack varied between mere tremor of the head, a partial paralysis of one limb and complete paralysis of legs and arms. The paralysis had in some instances nearly or completely disappeared and in others it had become reduced but still affected all the muscles of one or two limbs. In no instance did the second inoculation produce a frank renewal of the disease or appear to retard the progress toward recovery. Two animals seemed somewhat sick a few days after the second injection and soon recovered; it is not at all certain that the indisposition was the effect of the injection. The virus employed for reinoculation always produced infection in control animals.

There should be contrasted with this result the number of failures occurring after intracerebral inoculation in normal monkeys. Of eighty-three such animals, inoculated with emulsions of the spinal cord, all but six developed paralysis. Of the six failures, two animals are suspected of having suffered a mild attack unaccompanied by definite paralysis. Hence it can be concluded that in the period immediately following an attack of poliomyelitis and for a time thereafter, the monkeys are refractory to reinoculation with active virus. How enduring the immunity may be is still to be determined.

Of the six animals which failed to become paralyzed, three were

reinoculated. Two remained well and the third developed a severe paralysis. The last animal was reinoculated three weeks after the first injection, which period, we now know, is shorter than the longest limit of the incubation period, so that the result cannot be interpreted accurately. However, it appears that rare individual monkeys are highly refractory to infection.

Efforts have been and are still being made to induce active immunity through the employment of gradually increasing doses of active virus or of a virus modified by heat and other physical and various chemical agents. A certain degree of success has been achieved already and a greater success may be looked for. These results are reserved for a later report.

Experimental poliomyelitis develops, as a rule, quickly and after an incubation period of only a few days. It is not probable, therefore, that protective inoculation can be accomplished, by way of the subcutis, that shall prevent the onset of paralysis after an intracerebral inoculation. We have made an unsuccessful effort to achieve such protection. Emulsions of active spinal cord, warmed to 55° to 57° C. for one hour or to 60° C. for one half hour were injected beneath the skin at the same time that a usual intracerebral injection of virus was given. The two monkeys employed in the experiment developed paralysis in the usual manner.

VARIATION IN VIRULENCE OF THE VIRUS.

There is at present no reliable way of estimating the degree of activity of the virus since the number of organisms inoculated is not subject to control. The inoculated materials consisted of heavy suspensions in salt solution of the spinal cord, for preparing which portions from several levels were employed, or filtrates obtained from the suspensions, of which from two to four cubic centimeters were injected. Each of the two viruses has thus been passed through fifteen generations, in the course of which transfers it was observed that with each virus there occurred a sharp decrease of virulence at one point, namely, at the second generation of virus K. and the fourth generation of virus M. A. The change was indicated by feeble effects during several passages and final loss of power to transmit the infection. The strains of

virus, however, in parallel inoculations, retained their activity undiminished and serve, at the present time, to transmit the infection regularly and, we are inclined to believe, even more constantly than at first. Moreover, virus K. has, in the last generations, tended to produce the paralysis on the sixth or seventh day and virus M. A. on the eighth or ninth day.

MODES OF SPONTANEOUS INFECTION.

We have been successful in transmitting the virus through several avenues to the central nervous system of monkeys. The important question that arises relates to the avenue through which spontaneous infection in man takes place. In considering this phase of the subject, we have paid attention to the path of elimination of the virus into external nature, since such elimination must occur in order to maintain the virus alive and transmissible, and because of the possibility that the path of elimination may also be the portal of infection. In designing experiments to cover this point, we have had in mind the relation proven to exist between the infiltrations of the meninges and the meningeal vessels and the lesions in the cord and brain, and the fact that infection can be induced by intralumbar inoculation of the virus and the cerebrospinal fluid of paralyzed monkeys contains active virus. We also consider that the effectiveness of the intracerebral injections is in some way bound up with successful infection of the meninges. The brain matter, at the site of inoculation, does not show a marked specific reaction to the immediate presence of the virus.

The most direct connection existing between the meninges and the external world is by way of the lymphatics of the nasal and pharyngeal mucosa, through the cribriform plate. On account of its filterability, through which circumstance all bacteria can be eliminated, it has been possible to determine that the virus escapes from the meninges into the naso-pharyngeal mucosa. There still remains to be accomplished infection by the reverse route. The difficulty that presents itself in respect to this experiment is a common one in determining paths of infection in relatively non-susceptible species that shall reproduce those occurring naturally in man. The contributing factors, which act to promote spon-

taneous infection, are at present unknown. The observation on the infectivity of the naso-pharyngeal mucosa does not actually establish it as the site of infection in man or exclude the possibility of other modes of elimination of and infection with the virus.

VARIETIES OF ANIMALS SUSCEPTIBLE.

We have employed only the lower species of monkeys for our experiments. The greater number were *Macacus rhesus,* but all other species of old world monkeys that we used seemed equally susceptible. They included *M. cynomolgus, M. nemestrinus, Cercocebus fulignosus, Cercopithecus callitrichus* and *Papio babuin.* We also employed two species of new world monkeys, one belonging to the genus *Cebus* and including *Capuchinus.* The larger ring tail proved susceptible and the smaller did not, although five individuals of the latter were inoculated. The question whether the catarrhine are more uniformly susceptible than the platyrrhine species is an interesting one.

In this connection brief reference should be made to other species of animals employed for inoculation. Besides many guinea-pigs and rabbits, one horse, two calves, three goats, three pigs, three sheep, six rats, six mice, six dogs, and four cats have had active virus introduced in the brain but without causing any appreciable effect whatever. These animals have been under observation for many weeks. It remains to be discovered whether some of these species of animals may not after all be made to develop poliomyelitis by using directly spinal cord from many human cases, on the supposition that a virus of greater virulence may be found. We inoculated rabbits and guinea-pigs directly with each of the two specimens of human virus, and additional rabbits and guinea-pigs and the other animals with virus derived from monkeys.

DISCUSSION.

The studies[18] which we have made and presented here bring epidemic poliomyelitis among the well-defined infectious diseases and serve to explain some of its obscure features.

[18] Preliminary notes of these studies have been published in the *Journal of the American Medical Association* as follows: *Jour. of the American Med. Assn.,* 1909, liii, 1639; 1909, liii, 1913; 1909, liii, 2095; 1910, liv, 45; 1910, liv, 535.

It has now been shown that the cause of the disease is a minute organism that readily passes through Berkefeld filters and is demonstrated with difficulty under the microscope. Since no example of this class of pathogenic organisms is known to maintain a saprophytic existence, it can be assumed, for the present, that this virus does not lead a prolonged existence apart from its host.

The virus becomes implanted upon the leptomeninges, especially in the region of the spinal cord and medulla, where it sets up cellular infiltrative changes that are most marked in the perivascular lymph spaces of the arteries entering the nervous tissues. The vascular lesions constitute the primary causes of the lesions of the nervous system, the severity of which is determined by the particular vessels affected and the intensity of the involvement. The infiltrative lesions are confined to the perivascular lymph sheath and adventitia, but still other lesions must occur in the intima of the vessels from which the edema and hemorrhage arise.

The central arteries, entering the anterior median fissure and supplying the anterior gray matter of the cord, invariably show the infiltration and other changes described, through which the preponderance of affection of the anterior horns is accounted for (Plate XVIII, Fig. 1). Since the arteries supplying the posterior gray matter are less important, the lesions in the posterior cornua are slighter in spite of rich infiltration of the posterior spinal vessels. The gray commissure and Clarke's columns are affected to the extent to which their blood vessels are involved in the infiltration.

No part of the spinal cord is, as a rule, spared and the medulla probably never entirely escapes injury. But the degree of affection is determined by the richness of the arterial blood supply, whence is explained the liability of the lumbar and cervical enlargements to severe lesions. The irregularity in the branching of the central arteries probably explains the common variations observed in respect to affection of the two lateral halves of the body.[19]

The brain is far less commonly the seat of lesions but it is not

[19] Harbitz and Scheel, Path. Anat. Untersuchen über Akute Poliomyelitis und verwandte Krankheiten, Christiania, 1907, p. 194.

spared. Paralysis of the cranial nerves and especially of the facial nerve (Plate XIX, Fig. 4) follows upon them, but lesions also occur in parts of the brain which do not respond by paralysis. The brain injuries, like those of the cord, depend upon vascular lesions. Herpes was never observed in the monkeys, although the intervertebral ganglia were frequently infiltrated. The condition has been observed rarely and lesions have been described in the spinal ganglia in human beings.[20]

There are good grounds for believing that a considerable part of the paralyses, especially those that are not permanent, are the effects of temporary vascular impediments. The impediments are all outside the lumina of the vessels which are merely reduced in caliber through pressure; thrombi do not occur. Some of the functional disturbances are, possibly, thus anemic in origin, others are probably caused by slight degenerations and still others are undoubtedly caused by focal hemorrhages and edema. All these effects may, possibly, be recovered from: part by resolution of the cellular vascular infiltrate and reëstablishment of the lumen, part by absorption of edema and hemorrhage; and part by restoration of the mildly degenerated nerve cells. The severer degenerative and other lesions, through which actual necrosis is produced, do not become restored.

The lesions of the nervous tissue are highly variable in respect to the degree of degeneration, but remarkably uniform in respect to the character of the changes. Both the ground substance and the nerve cells in the gray matter are the seat of alterations: in the one there are edema, hemorrhage and cellular, lymphocytic chiefly, infiltrations; and in the other necrosis with disintegration of the nerve processes and cells. Polymorphonuclear leucocytes serve to disintegrate these and other degenerated cells, but play, on the whole, a very subordinate part. The white matter shows similar infiltrative and degenerative changes. Nuclear fragments occur in both places but not numerously. When the lesions are older, large numbers of vacuolated compound granular corpuscles and proliferated cells are present, and the proper elements of the nervous matter have disappeared.

[20] Harbitz and Scheel, *op. cit.*, p. 207.

A well-defined incubation period precedes the onset of the paralysis, but once the monkeys show symptoms of illness, the paralysis tends to appear abruptly. The prodromal symptoms and the concomitant symptoms are slight as compared with the spontaneous disease in man. The average incubation period in the monkey is about nine days, which is somewhat greater than that estimated for human beings. Wickman[21] believes that in human beings the incubation period ranges from one to four days, but he records examples in which it appeared to be as long as twenty-two and twenty-seven days.

The incubation period has been worked out on the supposition that the spontaneous disease in man is contagious. We observed no instance among our monkeys of a spontaneous transfer of the infection. However, we made no purposive experiments to test this point, and yet the inoculated and uninoculated animals were not kept carefully separate.

We have shown that the naso-pharyngeal mucosa contains the virus and yields a filtrate capable of setting up poliomyelitis. It can be assumed that the monkey is relatively insusceptible and hence difficult to infect through the natural channels, a fact already established for other infectious diseases. Spontaneous infection would, therefore, not be expected and more especially as monkeys rarely cough. Were the virus eliminated with the excreta, which has not yet been shown to occur, and were infection readily accomplished by way of the gastro-intestinal tract,[22] examples of spontaneous infection would conceivably occur more readily because of the usual soiling of the hands and food of the animals.

All the definite clinical types of poliomyelitis described in human beings have been observed in monkeys. Just what the significance of the abortive type so-called is may be ascertainable more readily in the monkeys than in man. The end results appear also to be identical: recovery may be complete or partial or death may result. Experimental poliomyelitis in the monkey is a severe and highly fatal disease and exceeds in the latter respect the spontaneous disease in man.

[21] Wickman, *op. cit.*

[22] Harbitz and Scheel, *op. cit.*

The literature on epidemic poliomyelitis in man is silent on the subject of reinfection and it does not appear that an attack of the disease has been noted to afford immunity from subsequent attack. We have made a number of attempts to reinfect, by intracerebral inoculation, animals which have recovered partially or completely from the first infection, but thus far without success. Hence it appears that immunity is afforded by an attack of epidemic poliomyelitis, with which observation we are inclined to believe experience with the spontaneous disease in man will be found to agree.

When our studies were begun, the only publication on experimental poliomyelitis in monkeys was that of Landsteiner and Popper.[23] Since our first results were published, the reports of other successful experiments have appeared. Thus Leiner and Wiesner,[24] Landsteiner and Levaditi,[25] and Römer[26] have employed successfully the intracerebral mode of inoculation of the virus. We shall not set down here a full transcription of their results but merely mention those which are of special interest in conjunction with our own. Thus Landsteiner and Levaditi independently ascertained that the virus can be filtered, and that the salivary glands are sometimes infectious; and Leiner and Wiesner found that the virus when injected into the small intestine, peristalsis being prevented by means of opium, or when introduced into the stomach by means of a catheter, sufficed to cause paralysis.

CONCLUSION.

The experimental study of poliomyelitis has yielded a large number of important facts relating to the spontaneous disease in man. The nature of the virus has been discovered, many of its properties have been ascertained, some of its immunity effects have been established, the clinical and pathological peculiarities of the disease have been elucidated, and a basis has been secured on which to develop measures of prevention.

[23] Landsteiner and Popper, *op. cit.*

[24] Leiner and Wiesner, *Wiener. klin. Woch.*, 1909, xxii, 1698; 1910, xxiii, 91.

[25] Landsteiner and Levaditi, *Compt. rend. Soc. de biol.*, 1909, lxvii, 592; 1910, lxvii, 787.

[26] Römer, *Münchener med. Woch.*, 1909, lxi, 2505.

ILLUSTRATIVE PROTOCOLS.

M. A. Virus.

Protocol A. Sudden onset, rapid progress and fatal termination of paralysis.—*Macacus rhesus* inoculated intracerebrally,[27] October 21, 1909, with emulsion of cerebral cortex. Well until 8 a. m., October 30, when it was noted that the animal got to his perch with some difficulty. The disability was rather indefinite. By 11 a. m. the four limbs were spastic; at 12 m. he could no longer get to the perch and a tremor of the body and nystagmus had developed. Attempts to move brought on marked incoördinated muscular seizures. 9 p. m. the condition appeared unaltered, but the handling required to take the temperature (which was 37° C.) induced collapse. Edema of the lungs set in and death occurred at 10 p. m.

Protocol B. Sudden onset and rapid progress of paralysis from subcutaneous inoculation.—*Macacus rhesus* inoculated with suspension of medulla December 20, 1909. The medulla had been kept for four days at room temperature. January 2, 1910, 8 a. m., paralysis of the neck, arms and legs present. The paralysis is not complete and the animal can stand, but unsteadily. 5 p. m. flaccid paralysis complete. January 3, animal weaker. Etherized.

Protocol C. Paralysis of cranial nerves, neck and limbs. Descending progress.—*Macacus rhesus.* November 18, 1909, inoculated intracerebrally. Until November 29, no symptoms. Then a tremor of the head appeared and the face and mouth were drawn to one side (facial palsy). The arms appeared weak, especially the right arm. November 30, the neck muscles are completely, the arms partly, paralyzed and the animal cannot stand upright. Etherized.

Protocol D. Paralysis of legs, weakness of arms, sudden onset and death.—*Macacus cynomolgus.* Intracerebral inoculation, October 28. Well but perhaps quieter than usual, November 5. November 6, 11 a. m., complete paralysis of both legs and weakness of both arms; sick. November 7, arms very weak but not paralyzed; animal very sick. Died 7.30 a. m., November 8.

Protocol E. Sudden onset of violent symptoms and death.—Baboon (*Papio babuin*). Inoculated intracerebrally with spinal cord November 16, 1909. No symptoms of illness until December 7, 9 a. m., when it was noted that the muscular movements were weak; no definite paralysis. About 2 p. m., the animal suddenly fell from the perch after which he was unable to stand. The sensorium remained clear, but attempts to move brought out marked involuntary muscular incoördinative movements. Once started, these movements were not readily controlled, apparently, voluntarily. Death occurred suddenly at 2.30 p. m.

Protocol F. Gradual onset of paralysis; partial recovery, resists second inoculation.—*Macacus rhesus* inoculated intracerebrally with spinal cord October 28, 1909. Well until November 9, when a tremor of body was noticed. Next day, when descending from perch the animal fell and it was then noticed that both legs were weak, the left more than the right. November 11, left leg paralyzed, right weaker. Arms remain strong. Animal only slightly sick. November 20. Left leg completely paralyzed, right weak. November 24, right leg becoming stronger. November 30. The right leg has recovered much of its strength and is

[27] All inoculations were made under ether anesthesia.

used in climbing; left leg limp. December 14, left leg has recovered so far that the residue of paralysis is noticeable only when the animal moves about quickly. Right leg normal. On reinoculation intracerebrally, no effect was produced.

Protocol G. Partial recovery; death from pneumonia.—Green monkey (*Arcopithecus callitrichus*). November 30, 1909, inoculated intracerebrally with spinal cord. Well until 8 a. m., December 10, when complete paralysis of legs was present. The paralysis did not extend, but pneumonia set in and death occurred, December 22. The lumbar enlargement of the cord showed large gray foci in the anterior horn.

Protocol H. Inoculation with blood.—Macacus cynomolgus. October 28, 1909, injected 2 c.c. citrated blood from paralyzed monkey intracerebrally. No effects followed. December 14, injected intravenously 25 c.c. defibrinated blood from monkey at onset of paralysis. December 31, paralysis of back, neck and left arm appeared. Etherized.

Protocol I. Failure of first followed by successful second inoculation.—Macacus cynomolgus. December 24, 1909, subcutaneous inoculation with spinal cord. No effect. January 3, 1910, repeated subcutaneous inoculation. 9 a. m., January 6, animal appeared well. 12.15 extensive paralysis present; cannot stand. Died at 12.30 p. m.

VIRUS K.

Protocol J. Intraperitoneal inoculation of human cord.—Macacus nemestrinus. Inoculated October 22, 1909. Well until October 30, when paralysis of left arm and right leg appeared. Paralysis rapidly increased hour by hour and involved both legs. Etherized.

Protocol K. Successful intraperitoneal inoculation of monkey's cord.—Macacus nemestrinus. Inoculated October 30, 1909, with 2 c.c. cord suspension from Monkey J. November 8, animal somewhat sick; weak in legs and arms. November 9, right leg and left arm completely paralyzed. Animal very sick. Cannot raise head. Etherized. Typical lesions in cord.

Protocol L. Successful subcutaneous inoculation.—Macacus rhesus. November 24, 1909, inoculated subcutaneously suspension of monkey's spinal cord. December 3, 9 a. m., animal appeared well and strong. 5 p. m. legs weak. December 4, 8 a. m. complete paralysis of both legs; weakness of both arms. Etherized.

Protocol M. Successful intraneural inoculation.—Macacus rhesus. November 14, 1909, injected 5 c.c. suspension spinal cord into sheath of left sciatic nerve. Next day no effect. November 21, well. November 23, 8 a.m., left leg completely paralyzed; right leg weak. 5 p. m., right leg paralyzed. November 24, arms very weak.

Protocol N. Successful intravenous inoculation.— Bonnet monkey. November 10, 1909, injected 2 c.c. suspension of spinal cord intravenously. November 18, 9 a.m., sick. Paralysis left leg; weakness right leg. 5 p.m. both legs almost completely paralyzed. November 19, arms weak. November 20, arms and right leg stronger. No change in left leg. November 29, recovery complete except for paralysis of left leg. Etherized.

Protocol O. Inoculation of filtrate.[23]*—Macacus rhesus.* December 1, 1909,

[23] The filtrates employed for inoculation were prepared from the original human spinal cord and from the cords of monkeys which were paralyzed. The filtrate is active also by way of the subcutaneous tissues.

intracerebral inoculation of filtrate from monkey's cord pressed through Berkefeld filter. December 5, 5 p. m., tremor of head and weakness of arms. December 9, 9 a. m., left arm paralyzed; 11 a. m., both legs completely and right arm partially paralyzed; neck muscles weak. Etherized. With the spinal cord obtained from this animal another monkey was infected.

Protocol P. Inoculation of glycerinated cord.—Macacus cynomolgus. An emulsion of monkey's cord kept in pure glycerine for 7 days was, after washing away the glycerine with salt solution, injected intracerebrally on November 16, 1909. November 24, animal quiet; next day legs weak. November 26, both legs paralyzed; arms weak. Etherized.

Protocol Q. Inoculation of frozen human cord.—Macacus rhesus. The cord was kept in the Frigo apparatus at $-2°$ to $-4°$ C. for 40 days and inoculated intracerebrally on December 1, 1909. December 8, animal not well. December 9, legs completely and arms almost completely paralyzed. Next day the arms and neck were completely paralyzed. Etherized.

Protocol R. Inoculation of human cord kept just above freezing temperature. —Macacus rhesus. The cord had been kept at about $+4°$ C. for 50 days, during which time it has softened and mould had overgrown its surface. Portions of the interior were inoculated intracerebrally on December 10, 1909. On December 23, the animal appeared sick and on the twenty-fourth, both legs were completely, and the left arm partly, paralyzed. Both arms and the neck finally became paralyzed. Death occurred on December 29.

Protocol S. Inoculation of cord dried over KOH.—Macacus rhesus. The cord had been suspended in a desiccator over KOH for 7 days. December 11, 1909, a subcutaneous inoculation was made. December 21, animal sick; left arm and right leg incompletely paralyzed. Next day paralysis of legs, arms and neck complete. Death at 11 a. m.

Protocol T. Inoculation into spinal canal.—Macacus rhesus. A suspension of the cord was injected into the spinal canal on January 18, 1910. Animal well until January 26 when both legs were first incompletely, then completely paralyzed. Etherized.

Protocol U. Inoculation with cerebrospinal fluid.—Macacus rhesus. This fluid, drawn by lumbar puncture at the onset of paralysis, was injected intracerebrally on January 23, 1910. Animal well until February 1, when paralysis, at first partial and later complete, of the legs and arms occurred. Etherized.

Protocol V. Inoculation with filtrate prepared from the naso-pharyngeal mucosa.—Macacus rhesus. The mucosa of the nose and the pharynx, including the tonsils, was dissected, rubbed up with quartz sand, macerated in salt solution, and pressed through a Berkefeld filter. The filtrate was injected intracerebrally on January 23, 1910. On January 31, the animal was sick and showed tremor of head and arms. The next day the legs and back were weak. February 2, the legs were paralyzed and the arms were weak. February 5, the arms had regained strength but the legs were still completely paralyzed.

Protocol W. Recovery of strength but persistence of paralysis.—Macacus rhesus. November 26, 1909, intracerebral injection of spinal cord from monkey succumbing to glycerinated virus. December 6, 9 a. m., left leg partially paralyzed. 6 p. m., both legs completely paralyzed. Next day arms weak and

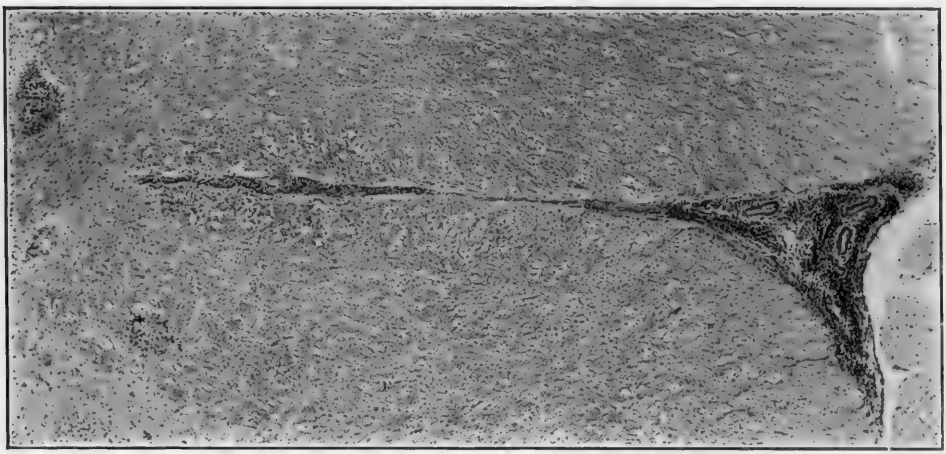


FIG. 1.

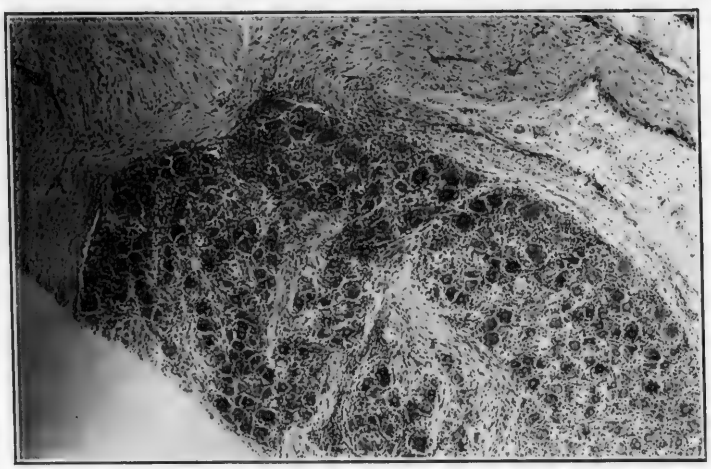


FIG. 2.

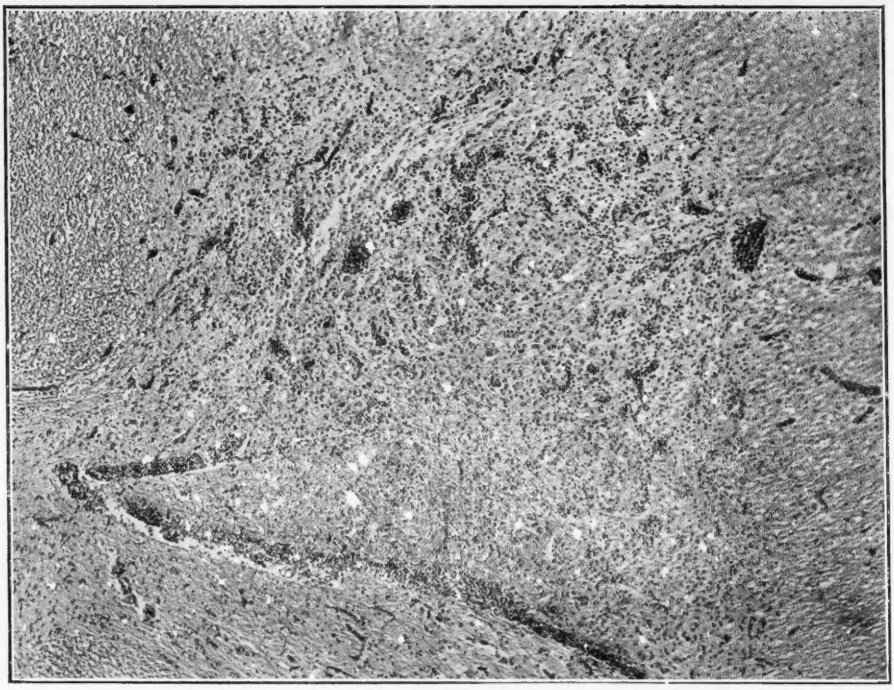


FIG. 3.

FIG. 4.

partially paralyzed. The animal was quite sick. Gradually the general health returned; the arms partly recovered strength; the body muscles remained strong; but the legs remained paralyzed and showed contractures.

EXPLANATION OF PLATES.

PLATE XVIII.

FIG. 1. Low power, showing pial and perivascular cellular infiltration.

FIG. 2. Moderate magnification. Showing diffuse and nodular round cell infiltration of intervertebral ganglion and adjacent nerve roots.

PLATE XIX.

FIG. 3. Medium magnification. Showing entire anterior horn degenerated and infiltrated with round cells; and extensive perivascular infiltration.

FIG. 4. Facial paralysis and deflection of the tongue.